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ERRATA, VOLUME V

Page 58, **line** at top of page should be transferred to top of p. 59.

Page 122, for *Fig. 2*, read **Fig. 3.**

Page 123, for *Fig. 3*, read **Fig. 2.**

Page 162, next to last line, last five words, should read "**will, we hope, be soon**"

Page 177, under Ludwig citation, for pp. 55–60 read **1–31.**

Page 216, line 20, delete *no.*, so as to read **160, 1905.**

Page 329, line 1, insert 1 before Coleosporium.

Page 395, for *Plates XXX-XXXI*, read **Plates XXX–XXXII.**

Page 418, plates *XXX, XXXI, XXXII*, change to **XXXIII, XXXIV, XXXV.**

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THE INFLUENCE OF ILLUMINATING GAS AND ITS CONSTITUENTS ON CERTAIN BACTERIA AND FUNGI*

C. A. LUDWIG

INTRODUCTION

It has been known ever since the observations of Girardin (6) in 1854 that certain phanerogams are susceptible to injury by the presence of illuminating gas in the soil or air. Since then considerable work has been done in both Europe and America on the question and on the allied one of the toxicity of smoke. One of the outstanding results of the later work has been a determination of the large rôle played by ethylene in the results observed and of the exceedingly small amount of ethylene which is necessary to bring about the reactions. It has been found, for instance, that the almost infinitesimally small amount of one part of ethylene in 2,000,000 parts of air causes closing of carnation flowers in 12 hours (1) and that the even smaller ratio of one part in 10,000,000 parts causes nastic curvatures in castor-bean seedlings (1).

With the bacteria and fungi, however, there have not been reported thus far any cases where such remarkable sensitiveness to the chemically more inert organic gases has been exhibited. In fact, very little has been done with these gases in this field; and, in most cases reported, gases were used in the pure condition very few or no attempts having been made to determine the lower limit of toxicity. It became, therefore, a matter of considerable scientific interest and some practical importance as affecting laboratory practice to determine as nearly as possible the lower limit of toxicity of illuminating

* Publication No. 167 from the Botanical Department of the University of Michigan.

gas and its separate constituents toward several of these organisms. The chief aim of this study was to make such determinations.

HISTORICAL

A number of investigations have been carried out to determine the reactions of phanerogams to low concentrations of different gases, and a smaller number to determine the more fundamental matter of the effect on the life processes. They have often been concerned in the first instance with smoke injury; and the results in general have tended to show, as would be expected, that mineral acid oxides or the oxides of toxic elements, as, for instance, arsenic, are decidedly toxic under conditions of much dilution in the air. They have also shown, as mentioned earlier in this paper, that certain plants show a remarkable sensitiveness to certain gases which are ordinarily considered to be quite inert. This work will not be reviewed further here, as it has only an indirect bearing on the problem investigated. A bibliography may be found in papers by Crocker and Knight in the *Botanical Gazette* (1, 2).

When we come to the lower plants, however, we find that comparatively little has been done along this line. Quite early in the history of bacteriology the question of the oxygen relation was worked out and methods were elaborated for its study with relation to any particular organism. This latter work involved a study of hydrogen and carbon dioxide as determining their availability for displacing the air in anaerobic culture conditions; but the matter of other gases, especially in less concentration than purity, appears to have seemed of little or no importance. There have been some pieces of work done, however, which have a suggestive or direct bearing on the problem and are therefore worthy of mention here.

Perhaps the earliest published paper of the kind was by Tassinari (15). In this case, the effect of tobacco smoke on several bacteria, including both pathogenic and non-pathogenic species, was investigated. The exposure to the smoke was made by means of a clever bit of apparatus in which a drop of the culture was held on a fragment of linen and the smoke drawn past it. The strip was then dropped into sterile medium and the time required for development noted. The check cultures developed uniformly in twelve to twenty-four hours. The smoked cultures with only one exception were delayed from twenty-four to one hundred hours in development or had failed to develop at all at the end of eight to twelve days.

Frankland (4, 5) investigated the effect of hydrogen, carbon dioxide, carbon monoxide, nitrous oxide, nitric oxide, sulphuretted hydrogen, and sulphurous anhydride on *Bacillus pyocyaneus* and the spirilla of Koch and Finkler. In carbon monoxide the spirilla produced colonies sparsely while *B. pyocyaneus* produced none until later exposed to the air. Nitrous oxide hindered the production of colonies, but did not prevent it, while nitric oxide, sulphuretted hydrogen, and sulphurous anhydride each prevented the development of colonies not only while the medium was exposed to the gas but also after its return to the air.

Krause (9) observed that *B. pyocyaneus* would grow in an atmosphere of illuminating gas or hydrogen sulphide but would not produce pigment under those conditions. When later exposed to atmospheric air the cultures produced the usual pigment.

Smith (14, p. 58) has made the statement that the small amount of carbon monoxide present where the oxygen has been removed by the potash-pyrogallol method of conducting anaerobic cultures is harmless to many bacteria, but that he has reason to think that it is injurious to others, even if it does not entirely inhibit growth. The grounds for his suspicion were not given.

Molisch (11) studied the effect of tobacco smoke on certain phanerogams and micro-organisms. He found that the movements of *Chromatium vinosum* (Ehrenb.) Winogradsky, *Beggiatoa* sp., and *Spirillum* sp. were stopped by the smoke. The growth of *Phycomyces nitens* was slowed down. His work showed that nicotin will not cause reactions in the phanerogamic plants used, *Vicia sativa*, *Pisum sativum* and *Cucurbita Pepo*, similar to those caused by the smoke, but that pyridin and carbon monoxide will each do it. This result is reinforced by the added observation that the smoke from burning paper, wood or straw will induce the same reactions.

Münz (12) has recently succeeded in isolating from garden soil, ditch water, river ooze and leaf fragments of various water plants certain bacteria which are capable of utilizing methane as a source of carbon and of energy. The writer regrets that he has not had the opportunity of reading Münz's paper. The note given above was made from an abstract in the *Zeitschrift fur Botanik*. The original paper is a dissertation at Halle, and, owing to the war, was not available for examination.

The published work with fungi and algae is even less abundant than with bacteria. A few pieces of work are worth mention, however.

Molisch has shown (11), as was mentioned above, that the growth of *Phycomyces nitens* is slowed down by smoke; and Thom (16) has reported that in an atmosphere of carbon dioxide no one of the species of Penicillium with which he worked showed growth within a week but that development set in after the tubes were restored to the air.

Richards and MacDougal (13), working with Nitella, found that this plant could live in carbon monoxide of 80 percent concentration but it was somewhat paler than the check in air.

Working with certain other algae, Woycicki (17, 18) has shown that illuminating gas will induce certain remarkable alterations both in the shape of the cell and in its internal structure. With species of Spirogyra, Cladophora and Mougeotia he found that in many cases curious outgrowths of the cells were produced which often resembled holdfasts, while the contents of the cells became more or less disorganized, according to the strength of the gas. The cells, in fact, were often killed; and the filaments usually became broken up into small pieces or even into the individual cells. *Cladophora fracta* var. *horrida* showed a much smaller degree of sensitiveness than Spirogyra. It was found also that the laboratory air often contains enough gas to induce alterations in the algae and that carbon monoxide and acetylene are capable of calling forth the changes.

Langdon (9, 10) has recently made the somewhat remarkable discovery that free carbon monoxide occurs in the floats of a Pacific marine alga, *Nereocystis luetkeana*, sometimes to the extent of 12 percent of the enclosed gases. The range was found to extend down to 1 percent, while the average was about 4 percent. This is interesting in view of the generally accepted belief in the poisonous nature of carbon monoxide to plants, since it shows that at least some plants capable of conducting photosynthesis contain tissues which are tolerant of quite large amounts of the gas.

INVESTIGATION

Organisms Used and General Methods

The organisms used in the study here reported consisted of bacteria and fungi. Of these, a number, *Bacillus subtilis* Cohn, *B. Kieliensis* (Lehm. and Neum.) Mig. ("ruber of Kiel"), *B. pyocyaneus* Gessard, *B. rubidus* Eisenberg and *Sarcina lutea* Schröter, were obtained from the department of bacteriology of the University of Michigan. A

number of others, *Bacillus carotovorus* Jones, *B. melonis* Giddings, *B. campestris* Pammel, *B. mycoides* Flügge, *B. solanisaprus* Harrison, *Pseudomonas radicicola* (Bey.) Moore, *Bacterium stewarti* Erw. Smith and *B. tumefaciens* Erw. Smith, were secured from the American Museum of Natural History through the botanical department of the University of Michigan. The following fungi were used: *Oidium lactis* Fresenius, obtained from the department of bacteriology, a strain of *Penicillium stoloniferum* Thom, isolated from moldy bread and determined by Miss Margaret B. Church, of the U. S. Department of Agriculture, a previously undescribed yeast,[1] isolated from the air at the University of Michigan, *Penicillium pinophilum* Hedgcock, *P. camemberti* Thom, *P. roqueforti* Thom and *P. expansum* Link, the last four of which were obtained from Miss Margaret B. Church through the courtesy of Dr. Charles Thom, and the four species, *Fusarium radicicola* Wollenw., *Gleosporium cingulata* Atkinson, *Endothia parasitica* (Murr.) P. J. & H. W. And. and *E. fluens* (Sow.) S. & S., which were received from Dr. Lon A. Hawkins.

The cultures were carried on ordinary 1 percent glucose, 1 percent peptone, 0.3 percent beef extract agar, with 0.5 percent sodium chloride and 1.5 percent agar, except that some of the experiments with *B. rubidus* were carried out on autoclaved potato slants. The color which *B. rubidus* develops on this substratum made the medium of

[1] I take this opportunity of thanking Dr H. W. Anderson for help with this species. He has made a taxonomic study of it and has kindly furnished the following diagnosis.

Cryptococcus Ludwigi H. W. Anderson sp. nov.

Morphology.—Cells round or oval, becoming elliptical in old cultures. Cytoplasm very coarsely granular. A single large granule usually evident. Buds arising from any point but usually from shoulders in elliptical cells. Size 3.5 × 4.5 μ.

Cultural characters.—On dextrose agar the streak is filiform, at first light pink, slimy, smooth, later becoming dry and very decidedly wrinkled and heaped. The dry, wrinkled type of growth is peculiar to this species of pink yeasts. On carrot and other solid media the streak has the same type of growth. In gelatin stab the line of puncture is filiform with no liquefaction.

Biochemical properties.—There is no fermentation of dextrose, lactose, galactose, sucrose, levulose nor raffinose. Litmus milk is rendered more alkaline.

From culture No. 51. Type specimen No. 51. Type slide No. 51. Cultures have been sent to several laboratories; and type slide, culture and dried material have been deposited in the herbarium of the University of Illinois. The organism was isolated from the air at the botanical laboratories of the University of Michigan, Ann Arbor, Michigan.

some value. The reaction of the agar varied with the different lots made up from nearly zero to slightly over + 1 on Fuller's scale, but was usually about + 0.8.

The exposures of the bacteria and some of the fungi to the gases were made in cotton-plugged test tubes confined in airtight chambers. This method has the disadvantage that it is practically impossible to get quantitative data by its means, such as could be obtained by using Petri plates and counting colonies, but the development can be followed better from day to day in tubes than in plates within a larger vessel. In most of the work it was a great advantage to be able to observe the cultures easily without removing them from the gas. The airtight chambers used consisted of four Novy jars and a number of bell jars with tubulature at the top which were fitted with perforated rubber stoppers holding tubes for the introduction of gas. Each bell jar was placed in a base composed of a heavy crystallizing dish with a layer of plaster of Paris about 2 cm. thick, impregnated with paraffin, in the bottom. The plaster of Paris was prevented from breaking the dish in setting by putting paraffined corrugated paper next to the wall of the dish in order to take up the expansion. The chambers were sealed by running melted paraffin between the case of the bell jar and the wall of the dish.

During the earlier part of the work, the gases were introduced from a Hempel gas burette by means of the pressure of a few centimeters of water. However, in most of the experiments the gas was allowed to enter directly and its amount was regulated by means of a mercury manometer. The reservoir for the mercury in this case was a wide-mouthed bottle closed by a two-hole rubber stopper through one hole of which the tube containing the mercury column passed. By means of a second glass tube through the other perforation in the stopper the apparatus was easily attached to any chamber in which the gas pressure was to be measured. When a certain amount of gas was to be introduced into a given chamber, the chamber and the manometer were connected at the same time to an aspirator and exhaustion was carried out, usually to about 15 cm. of mercury. The apparatus was then connected to the gas container and gas was allowed to enter until the pressure had risen the calculated amount on the scale, the calculation being on the basis that the amount of gas in a given volume varies directly as the pressure. The apparatus was then allowed to finish filling with air, after which it was closed

and set aside. The reason for introducing the gas while the pressure was low was to secure the vacuum as an aid to distribution in getting the gas through the plugs into the tubes in contact with the cultures. Some accompanying experiments with tobacco smoke and methyl iodide vapor, in which diffusion alone was relied on to get the gases into the tubes, gave results in the resulting cultures which showed that the gases did get into the tubes, at least in small amounts, very promptly. It seems not unreasonable, therefore, to think that the composition of the gas within the tubes approached pretty closely that in the rest of the chamber, although it was impossible, of course, to get absolute data on the point. The concentrations mentioned in all cases are to be considered not as exact values but merely close approximations. When pure gas of some kind was desired in contact with the cultures, one of two or three different plans was employed. In the case of illuminating gas, it was either allowed to pass through the vessel continuously during the experiment or it was passed through rapidly for one to two hours and then stopped. In the latter case it was usually renewed daily during the experiment. For other gases, which had to be manufactured for the purpose, the test tubes containing the cultures were fitted with perforated rubber stoppers through which small glass tubes passed. These test tubes were arranged in a chain and the gas was passed directly through them. The stoppers were sealed to the glass with which they were in contact by means of sealing wax, and the rubber connections between tubes were carefully wired and paraffined.

All results here reported, unless otherwise stated, were from at least two trials; and many of them were checked several times.

EXPERIMENTAL

1. Illuminating Gas

Source and Composition of the Gas

A large part of the work consisted of tests with illuminating gas. Such tests have the disadvantage, of course, that the gas is a mixture, and not a perfectly constant mixture at that; but its ready availability and the fact that it is the substance which usually contaminates laboratory air made it seem worth while to use it. The gas used was taken from the gas taps in the laboratories and was the same as

that used throughout the city of Ann Arbor. During the first part of the experiments (winter of 1915–'16) it was pure coal gas;[2] later (winter of 1916–'17 to Feb. 1), it was a mixture of coal and water gas; and at the last (after Feb. 1, 1917) it consisted once more of coal gas only, except for the 5-day period, February 12–16, during which time a small amount of water gas was mixed in. The gas before and after February 1, 1917, analyzed approximately as follows:

	Before Feb 1	After Feb. 1
CO_2	1– 2%	0.9–2.0%
C_nH_{2n}	4– 5%	3.5–4.5%
O_2	1– 2%	0.8–1.5%
CO	11–14%	6.0–7.8%
CH_4	25–30%	30– 35%
H_2	40–50%	35– 45%
N_2	about 10%	8– 11%

The figures given here are not the result of specific analyses made for the purpose of this study, but are the result of the examination of a large number of student analyses. However, as the gas was used at various times over an interval of a year and a half or more, it seems that a more exact analysis would be little or no more valuable for interpreting the results.

Ordinarily the gas was not washed. It was the original intention to do so, but this could not conveniently be done because the pressure in the pipes was too small to drive the gas through wash bottles. Moreover a few preliminary experiments with the gas showed that it did not exhibit extraordinary toxic properties toward the organisms used. It was therefore decided that as long as no very great toxic properties were shown it was not necessary to remove traces of H_2S, NH_3, or other inorganic gases which presumably might be present and exert harmful influences.

Effect on the Different Organisms

In giving the results of the tests with illuminating gas, and with the other gases as well, a brief summary will be given for each species used, instead of giving a chronological account of the experiments or of giving single experiments in detail.

[2] I am indebted to Prof. W. L. Badger, of the department of chemical engineering of the University of Michigan, and to Mr. Chas. R. Henderson, chemist to the Washtenaw Gas Co., for the analyses and data given here concerning the illuminating gas used in these tests.

Bacillus subtilis.—This organism was cultivated in the following approximate concentrations of illuminating gas: 0.5 percent, 5 percent, 10 percent, 25 percent, 50 percent, 75 percent, 85 percent, and 100 percent. In concentrations up to and including 25 percent, the colony development, both as to abundance and as to character, was practically identical with the development in air. This normal growth, as is well known, consists of a white, often wrinkled layer on the surface of the agar. The development in 50 percent gas and above, however, was quite different in character. The chief difference, and perhaps the only one of importance, was the much smaller mass of the colony produced. The colony was always very thin, so that it never had the opaque character of normal ones. The development was confined to the inoculated area and did not extend over the surface of the agar as was the case when development was normal. Occasionally only pin-point colonies were developed, or perhaps nothing at all until after return to the atmosphere. Complete sterilization practically never took place with an exposure not to exceed ten days; but it sometimes took a week or more for development to become evident after return to the atmosphere. In those cases where some development occurred in the gas it did not proceed further when returned to the air, but usually after the lapse of a variable period of time an area of normal development began at some point and grew over the slant. Inoculations made from these colonies grown in gas produced in the air a colony development differing very slightly or not at all from the normal in appearance.

Two series of experiments were run to test the ability of the organism to grow continuously in different percentages of illuminating gas. In these tests the inoculations after the first were made from cultures in the same concentration to which they were to be exposed unless no development had taken place in that concentration. In that case the inoculation was made from the highest concentration at which growth had occurred. In the course of this work, the organism was carried through 5 transfers in each of 5 percent, 10 percent, and 25 percent gas, 3 transfers in 50 percent, 5 transfers in 75 percent and 85 percent, and 9 transfers in pure gas. It is quite evident, therefore, not only that the organism can grow in the gas, but that it can continue so to grow for an indefinite time.

The growing of the organism in the pure gas seems to have caused little or no change in it, except in the colony character due to the

slowing down of growth to a very low point. This is evidenced by the fact, mentioned above, that a culture from a line previously carried in the air developed as well in the gas as another culture from a line previously carried in gas. It is also evidenced by the fact that in the first transfer into the air after a period of several transfers in gas the colony growth was normal in appearance in all respects. The examination of stained microscopic mounts supported the foregoing evidence. Bacteria from air cultures one day old and 11 days old and from a gas culture 11 days old were stained. In size, shape and especially in the absence of spores the bacteria of the gas culture (11 days old) resembled those of the one-day air culture more than they did those of the 11-day culture. There were only a few spores in the one-day culture, none in the 11-day gas culture, but most of the structures in the 11-day air culture were spores.

Bacillus pyocyaneus.—The culture of *B. pyocyaneus* used in this study developed the color only rarely. The color has therefore not been used as a character on which to base comparisons, although it has been so used in the past. The typical colony growth was rather dirty white, semi-translucent in character; and it was often difficult, because of the indefinite tint of the colony, to detect differences in the development of the growths under comparison. This organism was first grown in 5 percent, 50 percent, and 85 percent gas. The development was quite normal in 5 percent gas, but proceeded more slowly in the higher concentrations, so that whereas it took about three days for a culture in air to reach its maximum, it took two to three days longer for the 85 percent gas culture to reach the same stage. Attempts at this time to grow the species in pure illuminating gas met with three clear-cut failures and one apparent success, which, however, was possibly due to failure to displace all of the air in the container. In one of the cases of failure the organism developed (after a 4-day exposure) when returned to the air, but in the other two (after exposures of 6 days and 3 days) it did not develop within periods of 16 days and 27 days respectively. In later experiments, as will be shown presently, the attempt to cultivate the organism in pure gas resulted successfully. There was little or no alteration in the colony character in the gases, provided a colony was produced.

It was found also that the organism can apparently be carried indefinitely in most of the gas concentrations and perhaps even in pure gas. It was carried through 5 transfers in 5 percent, 10 percent,

and 25 percent gas, 3 transfers in 50 percent, 5 transfers in 85 percent, and 9 transfers in pure gas. In the unsuccessful trials with pure gas in the later work the organism always developed on the slant after being exposed to the air, although sometimes appearing in separate colonies instead of a streak, as if most of the inoculating bacteria had been killed. When success was attained in cultivating the organism in pure gas it then seemed likely that the bacillus had in some way developed the ability to grow under those conditions which were at first inhibitory; but this hypothesis was found to be untenable when trial was made by inoculating from a line which had been cultivated in the air only, for these cultures developed just as well as the ones which had been carried in gas for several transfers. These apparently contradictory results seemed quite unexplainable except on the assumption of a change in the composition of the gas, and it was thought at the time that no significant change had taken place. Later, knowledge was obtained of the variation in the gas concentration which has already been noted. Upon comparison it was found that the successful cultures of the organism began about Feb. 1, 1917, at the time the change was made to pure coal gas instead of the mixture of coal and water gas. The significant change in the composition of the gas would seem to have been the drop from about 12 or 13 percent to 7 or 8 percent of carbon monoxide. It should be remarked, however, as will be shown later, that neither of these concentrations of CO is of much significance if the rest of the mixture be atmospheric air. In fact, the tolerance of the organism to CO-air mixtures is so great that one would not expect a difference of only 5 or 6 percent in the concentration to exert any marked effect. It is also worthy of note that the first failure to grow in the gas occurred during the first period when the gas consisted of pure coal gas and had the lower carbon monoxide content. The results, therefore, are even yet unexplainable with the data at hand.

Bacillus Kieliensis.—This organism grows vigorously, reaching a maximum in 3 to 4 days, and has a very brilliant red color with a strong greenish metallic or coppery sheen on the surface. After the colony has reached its maximum the sheen gradually disappears, the colony becomes brickish red, and the pigment often diffuses more or less into the medium. This color responds readily to cultural conditions and so furnishes a sensitive index for detecting disturbances of the life processes. Its alterations are so numerous and complicated,

however, that no attempt will be made to describe them fully or to mention the many variations observed. In gas concentrations of 10 percent and less the development was normal. In 25 percent it was sometimes normal but more often slightly retarded and the color rendered less brilliant. In 50 percent gas the metallic sheen was usually nearly lacking, the color considerably lighter, and the rate of growth considerably less, so that it took 2 or 3 days longer for it to reach its maximum than it took in air. In still higher concentrations these changes were progressively more noticeable until pure gas was reached, in which surroundings development was very slight and the colony colorless or whitish, with only occasionally a trace of pink. In the gas-air mixtures the color was usually variable, ranging from deep red to light pink or whitish and usually with purple shades; and a number of these tints usually occurred in the same streak. The weak colonies which developed in gas of a high concentration grew vigorously and developed pigment when returned to the air, but the pigment never reached the depth of color shown by a colony grown in air from the start.

The organism was carried continuously through 5 transfers in 5 percent, 10 percent, 25 percent, and 85 percent gas, 3 transfers in 50 percent, 11 transfers in 75 percent, and 10 transfers in pure gas. Cultures in the air inoculated from cultures carried for several transfers in pure gas grew rapidly and developed abundant but not normal pigment, although the pigment production returned to normal after a few transfers in the air. Where the cultures had been carried in gas for only two transfers the color was normal at the first recultivation in air.

The examination of stained preparations failed to show any striking differences between the treated and untreated bacteria. In air culture the organism tends to show shorter, smaller, more coccus-like rods as the culture grows old. In gas the juvenile shape seems to be maintained for a longer period of time, probably owing to the slowing down of the development.

Bacillus rubidus.—Part of the cultures of *B. rubidus* in illuminating gas were made on autoclaved potato plugs. On this medium a clear orange color is produced which makes development easy to detect. The organism grew well in 0.5 percent and 5 percent illuminating gas; but did not grow in a strength of approximately 85 percent, except possibly in one trial, although it developed promptly when

restored to the air. In pure gas no development occurred during the exposure. In one case no development followed a 3-day exposure within 18 days after removal, and in another a 6-day exposure was followed by no development within 22 days, while in a third a very slight development began 5 days after the close of an 8-day exposure.

Sarcina lutea.—This proved to be one of the more susceptible organisms studied. It was, however, grown in 5 percent, 10 percent, 25 percent, 50 percent, 75 percent, and 85 percent of illuminating gas. There was ordinarily no checking of the development in the 5 percent concentration, but the growth gradually became less in all higher concentrations used. In all tests the air cultures reached their full development first, followed in succession by the others; but only rarely in 50 percent and above did the maximum in gas equal the maximum in air. The organism was carried continuously through 5 transfers in 5 percent, 10 percent, and 25 percent gas, 3 transfers in 50 percent, and 10 transfers in 75 percent. It was found impossible to secure unmistakable development in pure gas although there were a few cases of possible very slight development.

Oidium lactis.—*O. lactis* showed about average resistance to the effects of the gas. It was grown in all concentrations of illuminating gas employed, but its behavior in the stronger concentrations was rather erratic. Usually the growth under such conditions was very slight; and sometimes it started from only a few isolated points along the streak, as if the treatment had partially sterilized the slant; but at other times it would approach the maximum reached by a culture in air if left long enough. It was grown continuously for 5 transfers in 5 percent, 10 percent, and 25 percent gas, 3 transfers in 50 percent, 11 transfers in 75 percent, and 5 transfers in 85 percent and 100 percent. At times the character of the colony development in large percentages of gas differed from that in low percentages or in air, but here again the reaction was not uniform. In some tests the mycelium was more appressed and water-soaked in appearance in a high gas atmosphere and in others it was more upright and tufted or white velvety in appearance.

Cryptococcus Ludwigi.—This organism did not show any particularly remarkable characteristics in connection with these studies. Normally the colony is deep pink in color and is composed of quite a considerable mass of material. In gases the toxic effects are evidenced by a retarded or incomplete development of the colony and by a

paler color than normal. The organism grew in all the gas-air mixtures used—5 percent, 10 percent, 25 percent, 50 percent, 75 percent, and 85 percent gas—but not in pure gas. The 5 percent and usually the 10 percent concentration did not show any toxic effect, but at 25 percent of illuminating gas the development was always checked. At 75 percent and 85 percent the development was very slow and the colony quite pale, sometimes nearly colorless. Normally an exposure in pure gas was followed by development within a week when returned to the air, but in a few cases an exposure of a week or less seemed to sterilize the material, since no development had taken place in 13–18 days. The organism was carried through 5 transfers in 5 percent, 10 percent, 25 percent, and 85 percent gas, 3 transfers in 50 percent, and 11 transfers in 75 percent.

Penicillium stoloniferum.—This species grows rather rapidly on the medium used and soon becomes green with the large number of conidia produced. This color soon changes to some shade of brown, and later the colony is often overgrown with hyphae from underneath. The checking effect of unfavorable conditions can often be detected for several days after conidia production by means of the younger appearance of the retarded cultures as compared with the check. Development occurred in 5 percent, 10 percent, 25 percent, 50 percent, 75 percent, and 85 percent gas, but not in pure gas. It was quite normal in character to 50 percent but was much slowed down at that concentration. The checking of growth was observed at 10 percent but not at 5 percent. At concentrations of 75 percent and above development was slow and did not extend very far laterally, while a good many of the spores were apparently killed. As a result a cushion-shaped or roughly hemispherical mass of apparently upright hyphae was produced at each point of inoculation. The entire slant, therefore, often contained these pulvinate colonies, which sometimes reached a diameter of 2 or 3 mm. and became more or less confluent. Conidia were not produced under such circumstances except in one or two instances in which it is doubtful if the percentage of gas had been maintained. Even after the return of these cultures to the air there was only exceptionally any conidia production or other growth, although new cultures inoculated from them and kept in the air developed conidia normally. Cultures prevented from developing by being exposed to pure gas grew and produced conidia as usual in some cases upon being returned to the air. The species was maintained

continuously for 4 transfers in 5 percent, 10 percent, and 25 percent gas, 3 transfers in 50 percent, 10 transfers in 75 percent, and 4 transfers in 85 percent illuminating gas.

Bacterium stewarti.—*B. stewarti* is an aerobic organism which proved to be one of the most susceptible employed. It grew in 5 percent, 10 percent, 25 percent, 50 percent, and 75 percent of illuminating gas; and it was possible to keep it growing continuously in these; but the development in the last concentration mentioned was slow. A distinct checking effect was always shown at 10 percent and often a slight one seemed to be present at 5 percent. In the earlier work no development was secured at 75 percent, but later on growth did occur. The first development occurred at about the time the gas company ceased producing water gas, as was the case with the first development of *Bacillus pyocyaneus* in pure gas. The circumstance was probably due to the reduction of the CO content of the gas. No development was observed in any concentration used above 75 percent; but only rarely did development fail to take place after removal to the air, although it was usually 5–15 days in becoming visible and was also usually slight or very slight in amount.

The following organisms were tested once in each of 25 percent, 50 percent, 75 percent, and 85 percent, and twice in 100 percent illuminating gas.

Bacillus carotovorus.—With this organism there was slight development by the end of a 6-day exposure to pure gas in one test but none in the other at the end of an equal period. In both cases, however, prompt development followed a return to the air. Growth occurred in all the lower concentrations, but it was checked in all; and the retardation was still noticeable in the 25 percent concentration at the end of the 6-day period.

Bacillus melonis.—In one case with *B. melonis* the tube in pure gas showed a very slight development at the end of the period. In the other, however, no growth was visible although it became so soon after the return to the air. Growth took place in all the lower concentrations but it was much retarded in all.

Bacillus campestris.—With *B. campestris* also, development occurred in all the concentrations used except pure gas, although there was distinct retardation in even the 25 percent concentration. In pure gas there was no growth in one trial but a possible very slight development in the other. In both cases growth occurred after

removal from the gas, although it took periods of 6 and 5 days respectively for it to become discernible.

Bacterium tumefaciens.—There was visible development of *B. tumefaciens* in all percentages of gas except pure gas, although a distinct checking effect was observed in the lowest concentration used, 25 percent. No development took place in pure gas, but it occurred after returning the tubes to the air. The colonies in this case became visible in 5–8 days after the removal from the gas.

Bacillus solanisaprus.—There was a distinct and considerable checking of the development of *B. solanisaprus* in all of the concentrations of gas used, and in the greater ones the development was only slight. In the pure gas there was no visible growth, although it did occur following the return of the cultures to the air, in which case it became visible in 1–5 days.

Pseudomonas radicicola.—*Ps. radicicola* proved to be one of the more susceptible species. Development occurred in 25 percent gas, but it was only slight. At 50 percent concentration and above development was absent, although it occurred following the return to the air. The periods of time in which the colonies became visible in the cultures removed from pure gas to the air were 12 days and 5 days respectively.

Bacillus mycoides.—There was at least a very slight development of *B. mycoides* in all of the concentrations of gas used, but the 25 percent strength showed a slight retarding action, since it took 3 days for the colony to cover the slant from a spot inoculated in the center while in the air the slant was covered in 2 days. The development remained very slight in the higher percentages throughout the 6-day duration of the tests. The very slight colony developed in the case of the 50 percent and 85 percent gas, where the inoculation was by streak, was very similar in appearance to that of *B. subtilis* in concentrated gas. After being removed to the air normal development began in from 2 to 5 days and soon covered the slants. It did not originate all along the streaks, however, but at isolated points, so that separate colonies were formed, as if a partial sterilization of the slant had been produced by killing the inoculating bacteria between the points where the colonies arose.

A number of fungi were grown in Petri plates and the effects of illuminating gas noted, chiefly by measuring the diameter of the colonies at 3-day intervals (in some instances the sum of two different

TABLE SHOWING THE EFFECT OF DIFFERENT CONCENTRATIONS OF ILLUMINATING GAS ON THE GROWTH OF SEVERAL FUNGI

Species	Gas Conc.	First Trial				Second Trial				Third Trial			
		3d Day	6th Day	9th Day	12th Day[1]	3d Day	6th Day	9th Day	12th Day[1]	3d Day	6th Day	9th Day	12th Day[1]
Penicillium pinophilum	Air	14	28	43	58	6.5	24	41	56	9.5	20	35	56
	5%	—	—	—	—	7	24	39	53	10	23	35	50
	10%	—	—	—	—	6.5	21	36	51	8.5	20	33	49
	25%	10	19	29	—	4	16	27	42	8	18	30	47
	50%	8	13	22	—	2	11	21	36	4	10	17	30
	75%	4	5.5	7.5	21	2	4.5	7	22	4(?)	6	8.5	26
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium camemberti	Air	13	25	35	—	11	23	32	43	11	23	35	45
	5%	—	—	—	—	11	24	32	44	12	23	35	43
	10%	—	—	—	—	11	22	29	41	10	22	33	41
	25%	8	15	21	30	7.5	16	24	30	11	22	31	38
	50%	6	11	16	24	4.5	10	16	23	4(?)	10	16	25
	75%	4	5	7	—	2	6	9	18	4	6.5	9	17
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium roqueforti	Air	28	62	91[2]	91[2]	23	61	85	90[2]	25	53	79	90[2]
	5%	—	—	—	—	18	52	89	90[2]	23	48	80	91[2]
	10%	—	—	—	—	14	49	81	91[2]	23	46	78	90[2]
	25%	17	44	52	93[2]	7.5	—	59	90[2]	20	48	77	92[2]
	50%	8	29	40	70	3	12	23	73	6.5	13	20	53
	75%	2	3	5	33	1	4	8	37	5	6.5	9	34
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium expansum	Air	18	37	—	—	16	33	50	68	12	27	43	60
	5%	—	—	—	—	15	33	51	67	16	29	49	66
	10%	—	—	—	—	15	31	46	63	15	32	47	63
	25%	8	17	25	38	8.5	19	27	43	14	27	40	58
	50%	5	9.5	13	24	3.5	7.5	12	29	4	8.5	13	30
	75%	2	4	5	16	3(?)	5	7	22	3.5	4.5	7	24
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Endothia parasitica	Air	24	53	87	90[2]	4.5	34	65	88	12	38	68	90[2]
	5%	—	—	—	—	7.5	35	67	90	14	35	64	90[2]
	10%	—	—	—	—	7.5	33	60	83	11	34	62	90[2]
	25%	18	32	49	73	6	23	39	65	9	28	49	88
	50%	11	21	34	56	—	17	26	48	2.5	13	23	57
	75%	8	14	20	42	3	8	13	39	1.5	5	11	45
	100%	0	0	0	0	0	0	0	0	0	0	0	0

[1] The first three measurements in each case were made 3, 6 and 9 days respectively after exposure to the gas; the fourth was made 3 days after restoration of the cultures to ordinary air.

[2] Entire inside area of the plate covered with the colony, so that further growth was impossible. These values, therefore, are possibly too small, as the colonies may have reached the size mentioned before the close of the 12-day period.

Species	Gas Conc.	First Trial				Second Trial				Third Trial			
		3d Day	6th Day	9th Day	12th Day[1]	3d Day	6th Day	9th Day	12th Day[2]	3d Day	6th Day	9th Day	12th Day[2]
Endothia fluens	Air	21	55	92	—	8.5	43	73	86[2]	14	42	73	90[2]
	5%	—	—	—	—	11	42	71	87	8	28	54	83
	10%	—	—	—	—	7.5	29	51	73	11	32	56	87
	25%	9	12	34	60	5.5	22	39	67	7.5	22	38	71
	50%	7	9	21	45	1.5	10	17	37	0	9	25	42
	75%	1.5	6	11	30	0	2	6	27	0	2	7.5	34
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium radicicola	Air	24	45	66	90	17	47	73	95[2]	22	44	70	90[2]
	5%	—	—	—	—	15	44	71	90[2]	20	43	66	91[2]
	10%	—	—	—	—	14	43	70	92	19	42	64	88
	25%	23	44	65	87	14	41	66	85	18	40	62	86
	50%	20	42	63	87	12	36	60	82	14	34	55	81
	75%	16	39	54	76	7	23	38	59	5	25	40	66
	100%	0	0	0	0	0	0	0	0	0	0	0	0
Glomerella cingulata	Air	29	54	80	91[2]	20	55	84	88[2]	22	50	75	90[2]
	5%	—	—	—	—	19	52	80	88[2]	22	50	74	90[2]
	10%	—	—	—	—	17	50	78	88[2]	24	47	70	90[2]
	25%	18	37	58	82	12	39	68	90[2]	24	44	63	85
	50%	15	29	44	67	9	26	43	69	10	23	35	57
	75%	10	19	30	53	5	16	29	55	6.5	13	23	52
	100%	0	0	0	0	0	0	0	0	0	0	0	0

radii not in a straight line was taken instead of the diameter). The medium used was potato glucose (2 percent) agar (3 percent). It was poured into the plates and the fungus inoculated into the center of the freshly hardened layer of agar. The plates were then sealed under bell jars and the gas introduced in the usual way. The apparatus was taken down every third day to record data. In the case of the test with pure gas the gas passed constantly through the jar containing the cultures. This dried the agar in the first trial, and it was thought that the dry condition of the agar might have had something to do with the failure of the fungi to grow after the restoration to the air. In the second and third trials, therefore, the gas was passed over water in a bottle before entering the jar; and this prevented evaporation of the moisture in the agar.

The accompanying table records the organisms, the treatments, and the results obtained, the measurements being diameters of colonies in millimeters. It can be seen from this that the lower limit of toxicity, or at least considerable retardation of growth, is between 10 percent and 25 percent in most of the species; but in one, *Fusarium radicicola*, it is difficult to locate, and is higher than for the rest of the

species. None of the values indicating retardation at 10 percent or below is significantly smaller than the value for its check except for *Endothia fluens* at about 10 percent. The lower limit for retardation of *E. fluens*, therefore, would seem to be somewhere between 5 percent and 10 percent. The retarded cultures, after being returned to the air, grew at approximately the same rate as the regular air cultures, thus demonstrating that after-effects are usually lacking; except that no development occurred in any case in any plate which had been exposed to pure gas.

Some of the cultures, including the two species of Endothia and *Fusarium radicicola*, produced in the toxic amounts of gas a more compact, velvety, deeper colony, with the hyphae more erect than in air. It is likely that these hyphae have interesting morphological characteristics induced by the treatment, but opportunity was not found to investigate this feature.

It seems clear, therefore, that among the organisms studied, including 13 species of bacteria and 11 species of fungi, there is no example of the extreme sensitiveness to illuminating gas which is displayed by some phanerogams.

2. METHANE

Production and Purification of the Gas

It was found difficult to get methane of sufficient purity for the tests conducted. The best way to produce it is said to be to treat aluminium carbide with water; but owing to the war no aluminium carbide could be obtained. Some of the gas was prepared from methyl iodide by means of the copper-zinc couple. This gas was quite toxic to the organisms but had a distinct odor. It was therefore feared that some unchanged methyl iodide vapor passing over with the gas had been incompletely decomposed by the tower of zinc through which it had passed and that the reactions were due to this methyl iodide vapor. Some trials with methyl iodide vapor were accordingly conducted, and the results were corroborative of the fear just mentioned. The method finally settled on for preparing the gas with which most of the tests were conducted was the ordinary sodium acetate-soda lime method with barium oxide substituted for the soda lime. This method is said in some of the organic chemistry texts to produce nearly pure methane. In the earlier experiments

the gas was stored over water until required for use. Under these conditions the inhibitory effect of the gas was so slight as to create doubt as to its purity. Accordingly a sample was analyzed[3] and found to consist of a mixture of methane, hydrogen, oxygen, and nitrogen, of which the methane comprised about 50 percent and the nitrogen about 30 percent.

In order to get more exact data a mercury seal gasometer was secured for storage of the gas and each quantity of gas used was sampled for analysis. By this means dilution of the gas due to its solution in the water of the gasometer and the giving up of nitrogen to it by the water were avoided. It was also possible by means of the analysis to get an accurate measure of the concentrations used. The upper limit for the concentrations thus secured was 65 percent of methane with a maximum oxygen content of about 10 percent. A sample analysis follows:

CO_2	2.0%	CH_4	65.0%
C_nH_{2n}	0.5%	H_2	20.3%
O_2	10.3%	N_2 (difference)	1.9%

The results of the later tests confirmed the previously indicated low toxic properties of this gas as affecting the organisms in question. In fact, some of the previous tests gave rather stronger reactions on the part of the organisms than did the last ones in spite of the fact that all conditions indicate that the gas in the former trials was more dilute.

Effect on the Different Organisms

In these reports the results given are based on both the earlier and later work, care being taken not to make the gas seem less toxic than the data warrant.

Bacillus subtilis.—In all of the concentrations of methane used the development of *B. subtilis* was normal in character and good in amount. There was some checking of development in the higher concentrations which sometimes extended apparently as low as 25 percent (value uncorrected by analysis); but it was never great and was dissipated at the end of 3 days, except in a concentration of approximately 50 percent (corrected) or higher, where the slight retardation persisted in one or two cases for 6 days.

[3] The analyses of methane, carbon monoxide, and ethylene given in this paper were made by Prof. W. L. Badger and Mr. Philip W. Shepard, of the department of chemical engineering of the University of Michigan.

Bacillus pyocyaneus.—*B. pyocyaneus* grew well in all of the concentrations used, but with an almost indistinguishable retardation in percentages of 45 percent or above. This, however, had disappeared by the end of 3 days.

Bacillus Kieliensis.—The development of *B. Kieliensis* in methane was good throughout, but was slightly less vigorous in the greatest concentrations used. It was practically impossible, moreover, to determine the exact concentration at which the first inhibition could be said to occur. The color was also affected comparatively little, though it seemed in some cases to extend as low as 10 percent (corrected).

Bacterium stewarti and Sarcina lutea.—Contrary to expectations, these organisms were practically unchecked in all the percentages used except the very highest, about 60 percent (corrected); and even here the growth was good.

Oidium lactis.—The development of *O. lactis* was practically unchecked throughout the series of methane exposures. In the highest percentages, 50 percent to 65 percent (corrected), there was a tendency for the mycelium to have a white, velvety appearance instead of the more typically appressed, water-soaked appearance.

Cryptococcus Ludwigi.—The growth of this pink yeast was good in all concentrations of methane. It was usually slightly pale at 45 percent (corrected), somewhat pale and retarded at times at 50 percent (corrected), and distinctly pale and usually somewhat retarded at 65 percent (corrected).

Penicillium stoloniferum.—The growth of *P. stoloniferum* was slightly checked at concentrations of 45 percent to 65 percent (corrected).

As can be seen from the foregoing data, the toxic effects of methane on the organisms up to 65 percent of the gas are very mild and are certainly not of a character to suggest that 30 to 35 percent of methane in the illuminating gas used could be responsible for the inhibiting effect which illuminating gas exerts.

3. Ethylene

Production and Purification of the Gas

The ethylene used in these experiments was produced by heating 95 percent alcohol with c.p. sulphuric acid. It was passed through

wash bottles containing water, sodium hydroxide solution and c.p. sulphuric acid respectively. In the earlier work it was stored in a gasometer over water until required for use. The results were later checked by another experiment made with gas stored in a mercury seal gasometer. The following is the analysis of the gas used in this last experiment:

Acid gases, CO_2, SO_2, etc.	7.2%
C_nH_{2n}	81.0%
O_2	.3%
CO_2	.3% (or perhaps none)
Difference ($N_2 + H_2 + CH_4$)	11.2%

Effect on the Different Organisms

The first nine of the following organisms were tested in 0.4 percent, 4–5 percent, 20 percent, 40 percent, 50 percent, 60 percent, 85 percent, and 100 percent (uncorrected values), except as otherwise stated, of ethylene. The concentrations, corrected according to analysis, which were used in verifying the results were 4 percent, 8 percent, 20 percent, 40 percent, 60 percent, and 80 percent. The percentages mentioned in connection with the first 9 species in the following notes are the corrected values unless otherwise stated.

Bacillus subtilis.—With *B. subtilis* there was no abnormality of colony type and no great checking of development in any concentration. There was none at all below 40 percent, and it was doubtful at that percentage. At 60 percent, however, it was unmistakable although not great.

Bacillus pyocyaneus.—Owing to the semi-translucent nature of the colony of *B. pyocyaneus* it was difficult to detect small differences in development, so that the lower limit for retardation as given may be somewhat too high. There was very little inhibiting effect exhibited, however; and the type of colony was not altered. The lowest concentration at which a retarding effect could be clearly distinguished was 60 percent, with a possibility that a very slight inhibition occurred sometimes at 40 percent.

Bacillus Kieliensis. There was little inhibiting effect exerted by ethylene on *B. Kieliensis* The organism grew vigorously in all the concentrations, and was quite often as vigorous as in air up to a concentration of 85 percent (uncorrected), but usually retardation could be detected at 40 percent and color variations at 20 percent or even sometimes at 8 percent.

Bacillus rubidus.—The concentrations in which *B. rubidus* was tested were 0.4 percent, 4–5 percent, 85 percent, and 100 percent, uncorrected values. There were no cultures with analyzed gas for verification. In the first three percentages the cultures were on autoclaved potato, in the last on agar. It grew in all, and when growing on the potato produced the characteristic orange-yellow pigment freely.

Sarcina lutea.—This organism was tested in 4 percent, 20 percent, 40 percent, 60 percent, 85 percent, and 100 percent ethylene, uncorrected values; and the results were checked with the percentages mentioned at the beginning of this section. The lowest concentration at which inhibition occurred was 40 percent, but good growth occurred in all. The color of the culture was never much affected.

Oidium lactis.—This organism grew well in all the gas concentrations used, the cultures being scarcely distinguishable up to 60 percent from those in air. At greater concentrations slight inhibitive effects were noted.

Cryptococcus Ludwigi.—This yeast grew well in all of the tests although the development was checked and the color paler in the higher concentrations. The lowest percentage at which retardation was unmistakable was 40 percent. At 80 percent the growth was quite slow at first and the color nearly lacking. In a few days, however, the development became greater and the color darker, although not equaling that in the air until the return of the culture to the air.

Penicillium stoloniferum.—This species was tested in 4 percent, 85 percent, and 100 percent, uncorrected values, of ethylene, and the results were verified with analyzed gas as indicated above. The organism grew well in all concentrations but was slightly checked at 40 percent and increasingly so as the concentration increased.

Bacterium stewarti.—This species was tested in 50 percent and 85 percent, uncorrected values, of ethylene and the results verified with analyzed gas as reported above. It grew well in all concentrations used. In the early stages of the exposures there was inhibition at as low a percentage as 60 percent or perhaps at 40 percent but, by the third or fourth day the effect had disappeared in all.

The following species were tested, two trials each, in only 50 percent and 85 percent, uncorrected values, of ethylene. There was no confirmatory test with the analyzed gas.

Bacillus carotovorus.—The development of *B. carotovorus* while

exposed to ethylene was good in all cases tested. In 50 percent of the gas it was quite equal to the air culture and in 85 percent it was only slightly less vigorous.

Bacillus melonis.—In one trial the growth of *B. melonis* in both ethylene contents was about equal to that in air. In the other there was slight inhibition in the 85 percent concentration.

Bacillus campestris.—The tests seemed to show a slight inhibition of *B. campestris* at 50 percent of ethylene although they did not agree especially well on the point. At 85 percent the inhibition was somewhat greater but not at all remarkable.

Bacterium tumefaciens.—With *B. tumefaciens* the tests showed a clear inhibitive effect at both concentrations of the gas but greater at 85 percent than at 50 percent, and the effect was maintained until the cultures were removed to the air.

Bacillus solanisaprus.—The growth of *B. solanisaprus* in the two ethylene-air mixtures was practically equal to that of the same organism in air.

Bacillus radicicola.—The development of the check culture of *B. radicicola* in air and of the cultures in 50 percent and 85 percent ethylene were practically identical in both tests.

Bacillus mycoides.—In the first test with *B. mycoides*, where the inoculation was made in a streak, no difference could be made out between the growth in air and in the ethylene. In the second test, however, where the slant was inoculated at a single spot near the center, it took about four days for the colony to spread over the entire slant in 85 percent ethylene, and about three days in 50 percent ethylene, while in air the invasion was complete in two days.

It seems quite clear to the writer from the results mentioned above that the presence of 4–5 percent of ethylene in illuminating gas is totally inadequate to account for its effect on the bacteria and fungi studied.

4. Carbon Monoxide

Production and Purification of the Gas

The carbon monoxide used in these studies was made by heating crystallized potassium ferrocyanide with c.p. sulphuric acid and a little water. It was bubbled through sodium hydroxide solution to remove the small amounts of CO_2 and SO_2. In the earlier work it

was stored over water until desired for use. At the last, one experiment was run with gas which had been stored in a mercury seal gasometer and so had not been subjected to the alteration of composition due to absorption by the water and the giving up to it of gases in the water. The results of the last test were corroborative of the former ones. The analysis of the gas used in this last test is here given.

CO_2.....	1.0%	O_2 ...	1.2%	N_2 (difference)...	5.7%
C_nH_{2n}....	0.4%	CO ..	91.7%		

In view of the high percentage of CO in the gas used it has not seemed desirable to correct the values by calculation. The gas used in the earlier tests was probably lower in CO than that used in the last one. For this reason the results obtained in the last test have been used instead of the others where a variation occurred which seemed to be due to a higher CO content in the last quantity of gas.

Effect on the Different Organisms

Bacillus subtilis.—No effect of carbon monoxide on *B. subtilis* could be observed at 10 percent or below, but at 25 percent and above the same sort of very thin colony was produced as was produced in illuminating gas at the higher concentrations. As in the case of illuminating gas, also, the colony did not usually undergo change to the normal air type when returned to the air, but often a normal colony would start up at some point and invade the slant from that center. Using this organism as a measure of toxicity, therefore, carbon monoxide would appear to be something like twice as toxic as illuminating gas.

Bacillus pyocyaneus.—This organism grew in all the concentrations of carbon monoxide used although its development was slowed down to some extent in the higher percentages. It was difficult to determine any definite place at which the inhibition set in, owing to the indefinite tint of the colonies, although it seems likely that it should be placed at about 25 percent.

Bacillus Kieliensis.—*B. Kieliensis* was found capable of growing in all the test conditions with carbon monoxide. The first pronounced retarding effect occurred at 25 percent, although it was small and not altogether uniform. The color was sometimes paler at 10 percent and usually so at 25 percent, while at 75 percent and higher the

pigment was usually nearly or quite lacking and the development very slight. It will be noted that if we take *B. Kieliensis* as a test organism carbon monoxide appears to be just about as toxic as illuminating gas.

Bacillus rubidus.—In the case of *B. rubidus* a single successful series of cultures with carbon monoxide showed a retardation in the rate of development at 10 percent; at 25 percent it was quite marked; at 50 percent and 75 percent the development was very slight at the end of a 5-day period; and at 100 percent (uncorrected) there was no visible development. After return to the air vigorous growth took place in 2 to 3 days. This organism was not included in the supplementary test with the gas that was stored in the mercury seal gasometer.

Sarcina lutea.—The development of *S. lutea* was normal or nearly so to 10 percent of the gas but at 25 percent the inhibitive effect was clearly noticeable. At 50 percent to 75 percent the development was very slight during an 8-day period of exposure. At 100 percent (uncorrected) there was very little if any discernible growth.

Oidium lactis.—Carbon monoxide exerted a definite checking effect on *O. lactis* at a concentration of 25 percent and a possible very slight effect at 10 percent. The development was good at nearly 100 percent, however, and only slightly atypical in character. There was, however, a slight tendency in the gas for the hyphae to grow upward and assume something of a tufted character.

Cryptococcus Ludwigi.—This organism grew in all the percentages of the gas used, although slight inhibition occurred at 10 percent and the development was slight or very slight in the undiluted gas. With the inhibition of growth went also decrease in depth of color, so that in the case of the greatest inhibition the colony was practically colorless. The colonies in the gas up to 75 percent reached a maximum quite as great as that in the air but took a few days longer, while for colonies in an atmosphere containing more carbon monoxide neither this maximum nor the typical intensity of color was reached. Upon return to the air, however, these conditions were attained.

Penicillium stoloniferum.—The lowest CO content at which growth was checked was 10 percent. At this percentage the checking was very slight, but increased with increase in the CO content, so that growth was very slow at 75 percent and above although conidia were usually produced, and if not they followed promptly on return of the culture to the air.

From these results it would appear that carbon monoxide is approximately equal to illuminating gas in the inhibitive effects on the organisms tested and considerably more active in this way than either methane or ethylene. It would not seem to be sufficiently toxic, however, to be the sole cause of the effects produced by the illuminating gas.

5. General Observations and Discussion

It was noted in the present work, as was of course to be expected, that not all organisms showed the same sort of reaction or exhibited the same degree of tolerance to the gases. Thus *B. subtilis*, *B. pyocyaneus*, *B. mycoides*, and *B. Kieliensis* showed a high degree of tolerance for illuminating gas. In the case of *B. subtilis*, *B. mycoides*, and *B. Kieliensis* the colony in the high concentrations was quite different in appearance from the normal one. In the case of the first two species this is owing perhaps merely to the very small mass of material produced, but in the last named it is associated also with a decrease in pigment production. However, in the case of *B. pyocyaneus* and *O. lactis* the appearance of the colony was comparatively little altered. Probably the most sensitive of the species studied were *Sarcina lutea*, *Bacterium stewarti*, and *Penicillium stoloniferum*. Among the group of fungi tested together, *Fusarium radicicola* was the most resistant while *Endothia fluens* was most inhibited by the lower percentages of gas, followed by *E. parasitica* and *Penicillium pinophilum*.

The data do not seem to warrant any conclusion that any of the strains acquired an increased degree of tolerance for illuminating gas by being cultivated continuously in its presence. Such did seem to be the case for a time with *Bacillus pyocyaneus* and *Bacterium stewarti*, but when cultures inoculated with the original mother strain, which had not been exposed to gas at all, were exposed to the gas along with the supposed acclimated strain, the development of the unacclimated strain was quite equal to the other. In fact there was some evidence that continuous growth in toxic concentrations of the gas weakened the organism slightly. This was more clearly evidenced with *Bacillus Kieliensis*, perhaps, than with any other and was shown by the fact that the color production was not quite normal for three or four transfers in the air after several transfers in pure gas.

It is hardly possible at this time to state definitely just what causes the inhibiting action of illuminating gas. Some checks run with hydrogen, carbon dioxide, and air washed in pyrogallol indicated rather strongly that a good part of it is due to the lack of oxygen, even with the facultative anaerobic species. Not all of the results can be so accounted for, however, as it does not explain the after-effects, nor why one gas in a given concentration should produce a greater effect than another, as, for instance, why a mixture of 25 percent carbon monoxide and 75 percent air should produce almost as great a retarding effect on the growth of *B. subtilis* as a mixture of 50 percent illuminating gas and 50 percent air. Certainly no one component of the illuminating gas has toxic properties sufficient to account for the results. Ethylene and methane are relatively innocuous, and in addition ethylene is present in only small quantities in illuminating gas. There is also to be considered the possibility that some of these compounds, especially methane, may serve as food material for the organisms. In this connection the work of Münz (12) is suggestive since it shows that some bacteria are capable of assimilating methane. Carbon monoxide proved to be more toxic than the illuminating gas in some cases, but it also is present in only small quantities. What appears to be the most reasonable hypothesis for the present is that the results are the sum of a relatively large effect due to the dilution of the oxygen plus a smaller effect due to the weakly poisonous properties of some of the component gases, the most important apparently being carbon monoxide.

In view of the very great toxicity which illuminating gas and ethylene show toward many phanerogams, it was a distinct surprise to the writer to find his cultures showing uniformly such a high degree of tolerance to these gases. The comparative degrees of tolerance can perhaps be better realized by a brief consideration of the general results for the two kinds of plants. One of the recent studies on the effect of illuminating gas and its constituents on some phanerogams and higher green cryptogams (*Doubt*, 3) included a large number of species in the plants tested. The concentration of gas at which reactions first occurred in some of the most sensitive species was 25 parts per million (0.0025 percent), while the only species not affected at 60,000 parts per million (6 percent) were species of Polypodium, Aspidium, and Asplenium, although some others which were affected could live at that concentration. In all of the phanerogams tested

at this degree of concentration reaction was obtained. In the case of the 25 cryptogams reported in the present paper, however, there was uniformly no visible reaction at 5 percent. So far as present records go, therefore, the phanerogams which are most tolerant to illuminating gas are not more tolerant, indeed are apparently less so, than the most sensitive bacteria and fungi. In other words, the least sensitive phanerogams are more sensitive than the most sensitive bacteria and fungi. It was also a matter of surprise, in view of the extreme toxicity of ethylene to phanerogams, to find that it is relatively innocuous to the cryptogamic species studied. In this case carbon monoxide was found to be considerably more toxic than any other organic constituent of illuminating gas. The fact that ethylene is more toxic to one group of plants and carbon monoxide to the other is further evidence of a great difference in the sensitivity of the two groups.

Incidentally it may be remarked that so far as the results from the species studied in this investigation can be projected to cover all species, they indicate that there is only a small chance that the gas which would escape from the gas fixtures in a room would be enough to invalidate results obtained from cultures in that room. It should be remembered, however, that even phanerogams vary considerably in this regard, as has recently been shown by Miss Doubt (3) and others; also that at least some algae appear to be quite sensitive, as is reported by Woycicki (17, 18). It would not be at all surprising, therefore, and is perhaps to be expected, even, that some more sensitive bacteria and fungi will yet be found. The known existence of only a few such would render precautions necessary in bacteriological and mycological work which in the light of the results here reported seem unnecessary.

CONCLUSIONS

1. None of the species of cryptogams studied, including 13 bacteria and 12 fungi, shows any very marked sensitiveness to small amounts of illuminating gas or its components.

2. In the higher concentrations (25 percent and above) of the gas and its components, however, most of the bacteria and fungi used are checked in growth or wholly stopped. In the latter case growth will usually take place after exposure to the air, although often from a comparatively few foci, as if many of the cells had been killed. Sometimes the culture is entirely sterilized.

3. Different species exhibit different degrees of tolerance for the gases, and in general a species which is relatively intolerant of one is relatively intolerant of others.

4. There was no real evidence that the continued culture of an organism in illuminating gas induces the development of an increased tolerance for the gas by the strain so cultivated. On the other hand there was some slight indication that the vigor of a strain so cultivated is slowly lowered.

5. The colony habit of organisms is often modified more or less strikingly in the more toxic gases. This is exemplified especially in the color variations of *B. Kieliensis*, the decrease in colony mass and gross appearance in *B. Kieliensis*, *B. subtilis*, and *B. mycoides*, and the more compact, upright arrangement of hyphae in several fungi.

6. Ethylene and methane are relatively less inhibitory to the organisms used than is illuminating gas, but carbon monoxide is about equal to the illuminating gas in this respect.

7. The effect of the gas cannot be laid to any one constituent, but is probably the sum of the small effect of each plus the greater effect of a deficient oxygen content.

8. Incidentally the foregoing results indicate that the amount of illuminating gas often present in laboratory air is not a menace to scientific results in bacteriology and mycology.

The writer takes pleasure in extending thanks to Professor F. C. Newcombe for suggestions and help tendered during the prosecution of this study, and also to all others who have helped in forwarding it.

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A BACTERIOLOGICAL METHOD USEFUL FOR THE STUDY OF OTHER MICRO-ORGANISMS

FREDA M. BACHMANN

We have been using in some studies of bacteria for the past two years a method which appears to be equally valuable for the study of other micro-organisms. The bacteria are grown in a thin film of medium upon a microscopic slide and later stained. It was devised by Frost[1] in his studies on the bacteriology of milk in order to obtain a more rapid method of counting living bacteria in milk than the ordinary or standard plate method. It is invaluable not only to determine quickly the number of organisms in any richly seeded material, but also for a study of the morphology of the single cells or of the colonies. Very beautiful preparations may be obtained which show the relation of the cells to each other as well as the cytology of the individual cells. Recently I have applied this method slightly modified to the study of the yeast cell with very gratifying results.

Thaxter's potato hard agar, which consists of potato broth with 3 percent agar and 2 percent dextrose, is a favorable medium for the growth of yeasts. It is important that the medium be very clear. This is accomplished by the addition of egg albumen, followed by boiling and filtering according to the usual methods of procedure in the making of media. In this way it is possible to obtain a clear amber liquid which becomes only slightly less clear as it solidifies. This is introduced in approximately 5 cc. amounts in test tubes and sterilized.

When the plates are made, the slides are first thoroughly cleaned to free them from grease or other adhering material, then sterilized in the flame from a Bunsen burner and then placed on a warm stage. The temperature of the slides should be somewhat above the solidifying point of the medium used. The agar is liquefied and cooled to not lower than 45 °, then inoculated with about 1/10 cc. of a rather heavy suspension of yeast cells in water. These are evenly distributed in the medium

[1] Frost, W. D. A Rapid Method of Counting Living Bacteria in Milk and Other Richly Seeded Materials. Jour. Amer. Med. Assoc. **46**: 889–890. 1916.

by drawing up the fluid into the pipette several times. Approximately 1/20 cc. of the inoculated agar is placed on a slide. Another slide is placed on top of it, so that about three fourths of the surface of the slides are in contact. This spreads the medium quite evenly on the two slides. They are then drawn apart and at once placed in a moist chamber. The agar solidifies in a very few minutes. When only a few slides are prepared, I have found it very convenient to place them in Petri dishes which are lined with wet filter paper. In so small a container the atmosphere becomes saturated with moisture in a very few minutes and later the agar film shows no indication of drying. If very many slides are prepared it may be found more convenient to use some device like the specially prepared cabinet described by Frost.[2]

The slides in the moist chambers are incubated until the colonies have grown to the desired size. The cells multiply rapidly, resulting in colonies of various sizes. The films are then ready to be fixed and stained. In the process of fixing, washing with water, staining, dehydrating, etc., it will be necessary to exercise great care that the delicate film of agar does not float off the slide. For fixing the cells I have used picro-formol and Flemming's weak solution diluted one half. The slides are placed in a staining jar with the fixing fluid cover-

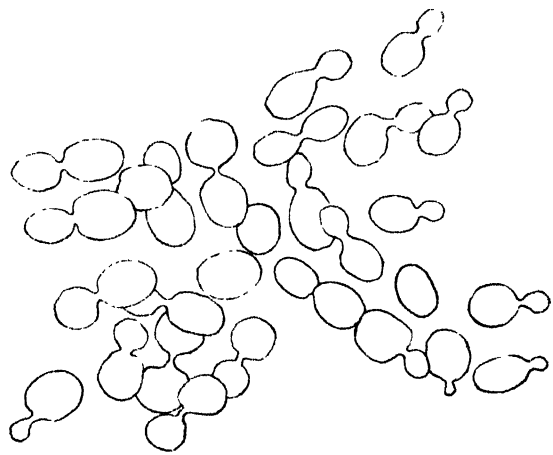


FIG. 1. Yeast colony, grown, fixed and stained on microscopic slide.

[2] L. c.

ing about half of the slide. This allows a part of the agar film, which is above the liquid, to dry firmly on the slide and so helps to prevent loss of the film. If the entire edge of the film becomes loose from the slide, the film is certain to be lost. I have introduced and removed all liquids rather slowly and very carefully with a pipette in order to prevent a rapid movement of the liquid from tearing the film. Sometimes a portion of the film will be torn off, but more often the film remains attached to the slide throughout.

After fixing, the films are washed and then stained. I have used Heidenhain's iron hematoxylin with excellent results. After staining,

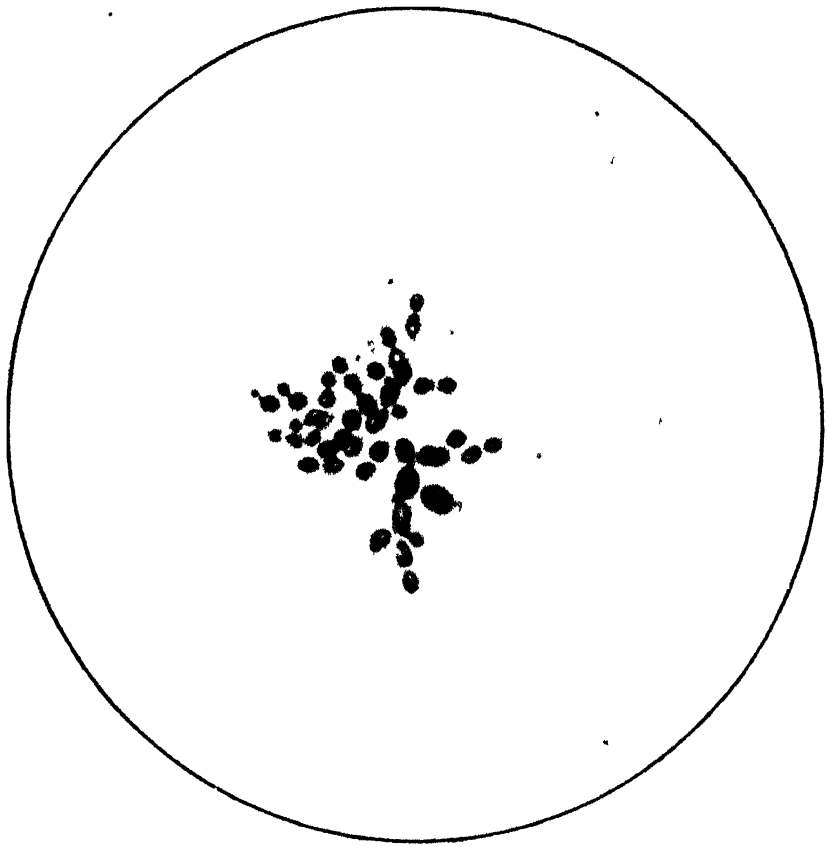


FIG. 2. Microphotograph of a colony of yeast, grown, fixed and stained on a microscopic slide.

the slides are washed in water, dehydrated with alcohol, using first a very dilute solution and lastly absolute alcohol, then rinsed in xylol and mounted in Canada balsam. The colonies are flat. This is especially desirable for a cytological study, because the cell structure is not obscured by anything in a higher or lower plane. The accompanying text figure and microphotograph illustrate the smaller colonies on plates which have been incubated nine hours at 37° C. and stained with Heidenhain's iron alum hematoxylin.

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THE DEVELOPMENT OF SOME EXOGENOUS SPECIES OF AGARICS

GERTRUDE E. DOUGLAS

In 1914, while looking for agaric material suitable for developmental studies, abundant young stages of several exogenous forms were found. At that time Professor Atkinson pointed out the need of a thoroughly modern investigation of these forms, both on account of the incompleteness of the early work and because the method of gill development in the endogenous forms was at that time receiving considerable attention. It seemed important to determine if the method of gill formation of species, differing in the presence or absence of a universal veil but otherwise rather closely related, would show any very radical differences in the method of the formation of their gill salients.

Until the work of Blizzard (9) in 1916, no study of development of exogenous forms of agarics had been made with our modern methods of technique. A considerable number of exogenous as well as endogenous forms[1] were early studied by Hoffmann (17, 18, 19) and among them one species of *Entoloma* (*E. sericeum* Bull.). Although his work was of necessity somewhat limited, he came to the correct conclusion with regard to the differentiation of the hymenophore primordium in the groove formed between the stem and pileus fundaments, which had been formed by the epinastic growth of the pileus hyphae. He also noted the formation of an even palisade layer before the appearance of the gill ridges.

Four exogenous forms, among them being one *Mycena* (*M. vulgaris*), were studied by DeBary, whose final conclusion (13, 14) with regard to the formation of an even palisade layer, preceding the development of the gill ridges, agreed with that of Hoffmann (17, 18, 19). In addition he emphasized the order of their development in a centrifugal manner from the stem to the margin of the pileus.

In 1889 a great number of forms were studied by Fayod, but owing

[1] For a complete list of the early forms studied see Atkinson, G. F., Origin and development of the lamellae in *Coprinus*. Bot. Gaz. 61: 89. Footnote 1, 1916.

to his rather indefinite limitations of the "couche piléogène" and the "primordial cuticle," and also to the fact that he considered all the forms to arise endogenously, there is considerable need for reinvestigating these forms.

The excellent work of Blizzard (9) in 1917 has confirmed, in the case of certain species of *Omphalia*, *Clitopilus* and *Clitocybe*, the final conclusion of DeBary and Hoffmann. This present paper is offered as a further contribution to our knowledge of the exogenous forms.

MYCENA SUBALCALINA Atkinson.*

(Figs. 1-24)

One of the autumnal species of fungi, which is found frequently in great abundance in the woods about Ithaca, N. Y., is *Mycena subalcalina*. It is a small mushroom, rarely exceeding 2 cm. in the diameter of its pileus. On account, however, of the profusion in which it grows on decayed stumps and the ease with which it may be collected free from soil particles, it lends itself very readily to morphological study.

Collection and Preparation of Material.—The material for this study was collected at two different times, once on November 7, 1914, from the marsh at McLean, N. Y., and again on January 20, 1915, from the inside of a hollow trunk in the Beech Woods by Six Mile Creek near Ithaca, N. Y. At this time the ground was covered with snow and the fruit bodies, which were exposed on the surface, were covered with a coating of ice, apparently interrupted in their development by the coming on of winter. Some of these latter, together with others from the interior which were not frozen, were immediately fixed in medium chromo-acetic acid. All of the material proved

* **Mycena subalcalina** Atkinson, n. sp. Caespitosa vel subcaespitosa, 3–7 cm alta; pileo convexo dein expanso, avellaneo-brunneo vel castaneo-brunneo, 1–2 cm. lato, leniter striato, lento, humecto sed non viscido; lamellis albis demum subsordidis, angustatis, subdistantibus, arcuatis, adnatis, dente decurrentibus; cystidiis nullis vel raris, ad aciem lamellarum clavatis, non emergentibus, frequenter mucronatis; sporis quaternis, levibus, subellipsoideis vel ovalibus, minutissimis, 3 6 × 2–2 5 μ; stipite sursum albo vel pallido, deorsum luteo-rufescenti, ad basem atrobrunneo et strigoso, apice leniter pruinoso, 2–3 mm. crasso.

On decaying wood in the vicinity of Ithaca, N. Y. The plants are tough and pliant when fresh, with an alcaline or nitrous odor, though sometimes faint.

satisfactory, the freezing apparently not affecting the buttons. After washing and dehydrating they were cleared in cedar oil and embedded in 52° paraffine. Sections were cut from 5–7 microns in diameter and stained with fuchsin.

Early Stages in the Development of the Fruit Body.—It was possible to obtain in this manner fruit bodies in which no differentiation had taken place. A longitudinal section of a young button, about 2 mm. in diameter, is shown in figure 1, attached to the base of a more developed plant. It consists of homogeneous tissue, composed of small, densely interwoven hyphae, about 1 μ in diameter. The external hyphae are somewhat larger (1.5 μ) and take the stain more readily. Later the young fruit body becomes somewhat flask-shaped, as shown by the section of the larger plant in figure 1. The interwoven hyphae assume a generally longitudinal direction throughout the center of the fruit body, but all over the surface they already exhibit a strong inclination to turn outward. At the apex this epinastic tendency has resulted in a differentiation into two regions, the pileus and stem primordia, separated by an annular constriction or furrow. The tissues of both regions, however, are still homogeneous, that of the upper, in fact, being made up of the extension of the hyphae of the lower.

Differentiation of the Primordium of the Hymenophore.—As the basidiocarp increases in size, the differentiation between the stem and pileus fundaments becomes more marked (fig. 2). As yet there is no distinguishable hymenophore fundament, although a slightly increased staining capacity of the hyphae in the furrow suggests that a rapid growth is taking place at this point. The elements are still similar to those which are characteristic of the margin of the pileus, broad (1.6–3.5 μ in diam.), and blunt at the ends (fig. 19). Very soon the typical hymenophore elements appear. They are slender and sharply pointed at first, as they are in many of the endogenous forms. They branch off very profusely from the subadjacent tissue of the pileus and are stained more deeply (figs. 3, 4, 5, 22).

Development of the Palisade Layer.—The dense crowding of these elements causes them to become organized very soon into a palisade layer. In figure 22, an enlargement of figure 5, we may observe that the under surface of this zone of very active growth is still somewhat irregular, due to the varying lengths of the narrow-pointed hyphae. Later they become more even and blunt and the fruit body presents

the appearance of figures 6, 17, 23. Compared with the thickness of the elements, this palisade layer is an unusually deep one. The hyphae become more and more crowded as a result of their rapid branching and their slight increase in diameter. For this reason the layer becomes very conspicuous in contrast to the looser, more inactive subhymenial tissue, from which these hyphae have arisen.

Development of the Gills.—Fruit bodies in the stage shown in figures 8–12 show the very earliest evidences of gill formation. We have seen that the palisade layer has become very crowded by the multiplication of its elements. As these continue to be formed, this extra growth produces a tension which must be taken care of in some way. This is accomplished by the growth downward of the subadjacent hyphae in regularly spaced radial rows, beginning at the stem and extending to the margin of the pileus. In figure 8 the palisade layer appears to be slightly decurrent on the stem. It is here that the salients first begin to form. These appear as two irregular folds in figure 9, which is reproduced from a section cut parallel to the median plane at the surface of the stem. As one passes outward, these folds become less marked (fig. 10) and finally disappear altogether. In figure 11, nearer the margin, we find the level palisade layer, while at the very outside of the pileus the irregular palisade primordium is present (fig. 12). The series of sections in figures 13–17, from a more mature plant, shows better-developed salients, while figure 18 represents a section through the margin. These salients are becoming more regular and, as the pileus broadens and the interstitial growth forces the primary salients apart, new secondary ridges make their appearance between the original ones (fig. 21). This method of gill development is then, in the main, the same as that described by Hoffmann (17, 18, 19) and DeBary (11, 12, 13, 14) in exogenous forms and also agrees with species recently studied by Blizzard (9). It is also similar to the method of gill origin in endogenous forms of the *Agaricus* type described by the earlier workers and in the modern work of Miss Allen (1), Atkinson (2, 3, 4, 5, 6, 7), Beer (8), Douglas (15), Sawyer (20) and Zeller (21), with the exception that in the endogenous forms, the ridges develop underneath in a more or less distinct "gill cavity," while in the exogeneous forms they are exposed from the first to the outside.

Development of the Pileus.—After the appearance of the annular furrow separating the pileus primordium from that of the stem, the

pileus grows very rapidly (fig. 2). Its texture is at first similar to that of the stem, since its hyphae, as mentioned above, are but the continuation of those of the stem which have bent outward under the influence of epinasty. By growth and branching new elements are being continually added, especially at the margin of the pileus. When the primordium of the hymenophore appears, followed by the level palisade layer with its compact row of parallel hyphae, the pressure causes the subhymenophore tissue to become stretched, looser and more open in texture. We noticed in the earliest stage a layer of more deeply staining tissue over the surface of the fruit body (fig. 1). It persists throughout the early stages and appears to be composed of enlarged hyphal cells (in fig. 6, 4 μ in diameter) which have been chemically changed or injured as they have grown through the substratum. They persist for a time in loose tufts, finally disappearing with the maturity of the plant.

Development of the Stem.—As the plant grows, the stem elongates rapidly and at the same time grows in thickness by the interpolation of new elements, which intertwine with each other but which take a general longitudinal direction. On the surface they bend strongly outward (figs. 1–13) instead of uniting to form a compact layer as they do in many forms with a smooth stem. The villous stipe of the young growing plants is due to this circumstance.

HYGROPHORUS

Collection and Preparation of Material.—Three species have been studied. The material used in the study of *H. miniatus* came from two different collections. Pieces of a decayed stump, containing young fruit bodies, were brought into the laboratory from McGowan's woods, near Ithaca, in October, 1915, and from these the young fruit bodies were chosen. Another collection was made by Mr. Blizzard at Seventh Lake, Adirondack Mts., N. Y., during August, 1916, from which the early stages in the development of the gills were obtained. The specimens of *H. borealis* were collected by Professor Atkinson and the author from rich leaf mold where numerous scattered mature plants were growing, in the sphagnum moor at Malloryville, N. Y., during August, 1914. *H. nitidus* plants were found growing in rich soil by the edge of Eighth Lake, N. Y., in August, 1916. The material of all three species was fixed in medium chromo-acetic

acid, cleared in cedar oil and embedded in 52° paraffine. Sections were cut from 5–7 microns in diameter and stained with basic fuchsin and carbol fuchsin.

HYGROPHORUS MINIATUS Fr.

(Figs. 25–47)

Young Stages.—Fruit bodies up to 1 mm. in length show no differentiation of tissues (fig. 25). The buttons are more or less conical and composed of compactly interwoven hyphae about 1.5 μ in diameter with conspicuous nuclei. A number of the fruit bodies, in about this stage of development, show, distributed throughout the tissue, other hyphae which are considerably larger in diameter and which have a strong affinity for the stain. They are possibly in the process of disintegration or are food-storage hyphae. They are also found in some of the later stages but have completely disappeared by the time that the hymenophore primordium is organized. It was suggested by Professor Atkinson that they probably function as nutritive elements for the other rapidly growing hyphae. The young buttons elongate very rapidly and become flask-shaped with long, pointed necks.

Differentiation of the Fundaments of Pileus, Stem and Hymenophore.—Later the fruit bodies undergo considerable broadening at the apex, leaving a decided groove, which separates the nearly oval pileus fundament from that of the elongate stem (fig. 26). In this latter region the primordium of the hymenophore later appears (fig. 27). The delineation of the pileus and the stem regions is not due to differences in the nature of their tissues but, as in the case of *Mycena subalcalina*, to the growth and rapid multiplication of the hyphae of the pileus fundament, which exhibit a strong tendency to turn outwards and downwards. Certain ones at the top of the stem and on the under side of the pileus show this inclination more strongly than the others and become directed outward in a nearly perpendicular direction to the tissues from which they arise. They are narrower than the elements at the pileus margin, have sharp ends, and become very numerous by successive branching. These hyphae constitute the primordium of the hymenophore (fig. 27).

Development of the Gills.—Differing from the condition found in the majority of forms, developing according to the *Agaricus* type, thus far studied, the appearance of gill salients precedes the formation of a definite, even palisade layer. While the elements of the primordium

continue to increase, the subadjacent tissue next the stem begins to extend downward in radial lines carrying the hymenophore primordium with it (figs. 28, 29 and 44). Gradually the palisade layer becomes more even (figs. 30–34, 47). By centrifugal growth the gill folds progress toward the margin of the pileus, where new primordial tissue is being organized (fig. 34). As in the case of *Mycena subalcalina*, the pressure of growth in the hymenophore region causes a loosening of the subadjacent tissue (figs. 29, 32, 47). More advanced stages in the development of the gills are shown in the fruit bodies represented in figures 35–39 and 40–42. As the gill ridges continue to grow, they become very broad with large spaces between. They finally assume the more or less triangular shape in cross section, which is one of the distinguishing characteristics of the genus *Hygrophorus* (figs. 41, 42). The trama, as can be seen in figure 40 in surface section and in figure 41 in cross section, is similar in character to that of the pileus.

The palisade layer, when finally organized, may be studied in photographs of figures 41, 45, 46. It shows especially well on the older part of the gills nearest the pileus. The ends of the hyphae have become very crowded and blunt, thus bringing about an even surface on the gill. No nuclei are present in the extremities but they are very conspicuous and deeply stained at the bases of the palisade layer cells. The adjacent tissue contains very abundant and deeply stained nuclei and presents much the same appearance as it did in species of *Cortinarius* (15). Just above this is the loose open zone which extends into the gills and into the pileus region between them, subadjacent to the hymenophore, and which gives rise by branching to the hyphal elements which make up the hymenophore. The palisade layer is not as compact (fig. 41), the hyphae have become clavate and the nuclei have migrated into the tips. These hyphal ends are apparently young basidia in the process of forming.

Structure of the Stem and Pileus.—The cap does not separate easily from the stem in mature stages, a fact which is due to the homogeneous character of the tissues of pileus and stem in gymnocarpous forms. It is made up of the continuation of the hyphae of the stem, as we observed in *Mycena subalcalina*. At the surface no blematogen is present and there is practically no change in the character of the tissue. The ends of the hyphae grow somewhat unevenly, so that the surface is at first irregular (fig. 28). Later on the ends become swollen and are cut off by cross walls (fig. 43).

HYGROPHORUS NITIDUS *B* AND *C*

(Figs. 48–66)

Young Stages.—In the earliest stage obtained of this form, a button about 5 mm. in length (fig. 48), differentiation of the pileus and stem regions had already begun to take place. In the photographs, the actively growing region appears by its deeply staining property to be at the tip, where the longitudinal hyphae are now multiplying profusely and growing outward in the formation of the pileus fundament. By the time development has reached the stage shown in figure 49, not only is the pileus primordium well delineated from that of the stem but we also have in the annular furrow between them the primordium of the hymenophore. Even though the whole surface of the fruit body is clothed with a layer of outwardly directed hyphae which take the stain deeply, this layer is differentiated by a still deeper stain and by somewhat smaller hyphae (about 2 μ in diameter as against those 3 or 4 μ at the margin of the pileus). These differences appear more sharply in an older stage (figs. 50, 51, 52). The hyphal ends on the surface appear to be disintegrating. This species is a viscid one and these deliquescing hyphae furnish the slime which covers the plant.

The Palisade Layer.—Although the hymenophore elements become crowded into a palisade-like layer, the surface of the gills never becomes smooth as it does in most of the endogenous forms studied and in the early stages of the two preceding species. Their hyphal ends are variously directed and uneven in length (figs. 53–57). This character is retained even in fairly mature stages (figs. 59–63, 66), whose surface in consequence is always uneven. The layer is, however, a very definite one and is homologous with the more even layers of the other species.

Formation of the Gills.—The first evidence of gill salients appears in the fruit body at a stage represented in figures 53–58, which shows the palisade layer being pushed out into very low undulations. In the next series (figs. 59–63), the gill character shows more clearly and it becomes quite evident that the method of formation is identical with that of *Hygrophorus miniatus*, previously described. The sections were taken from a fruit body which was growing close beside a second one of the same age, the margins of the two at the point of

contact having grown together (fig. 59). The trama of the gills has a very loose mesh which brings into sharp relief the deeply stained, crowded palisade layer, which covers the surface of the gills (fig. 59). In figure 66, an enlargement of the section shown in figure 61, the very uneven character of the palisade layer on the edge of the gills is apparent. The triangular form of the gill in section appears in the nearly mature plant of figures 64 and 65. The tissue of this fruit body is still homogeneous in character, except for the gills and the slime-producing layer over the surface. The portion of the pileus at this time is beginning to take on a hyponastic growth, causing the mushroom to become umbilicate.

HYGROPHORUS BOREALIS Pk.

(Figs. 67–80)

Early Stages.—The development of this species resembles that of *H. miniatus* and *H. nitidus* so closely that it will not be necessary to enter into its life history with as much detail. The youngest button found was nearly oval in shape and entirely homogeneous in composition (fig. 67). For a time after this the fruit bodies elongate very rapidly (figs. 68, 69), growing chiefly at the apex, as shown by the deeper stain in this region. At length, when they are well up out of the soil, the hyphal elements in the apex multiply and turn outwards in epinastic growth. Thus the pileus is formed (figs. 70 and 71). At practically the same time, the hymenophore primordium becomes organized (figs. 70, 71 and 72) in the annular constriction between the fundaments of pileus and stem. A very strong epinastic growth causes the margin of the pileus not only to bend downward but to inroll. Thus a protection is obtained for the young gill salients as they form. Before their appearance, however, a definite, even palisade layer is formed (figs. 73, 74 and 75).

Development of Gills.—Unfortunately very early stages in the formation of the gills are lacking. Figures 76-80 represent the youngest stage found. It corresponds very nearly to the stage represented in figures 36-39 in *H. miniatus* and shows the progressive maturity of the young ridges, as one passes from the margin (fig. 80) inward to the center (fig. 76). In figure 80 the dark stain represents the hymenophore primordium seen in surface view on the inside of the inrolled margin of the pileus. Figure 81 represents a very nearly mature

plant in radial section. The cut is made between the gills, but a portion of one gill joined to the stem appears in the photograph. The lightly stained area subadjacent to the palisade layer in figures 77–80 represents the layer from which the hymenophore elements have branched. This extends not only into the gills but also between them in the pileus region, subadjacent to the hymenophore. The corresponding region is shown very clearly by Blizzard (9) in *Omphalia chrysophylla* (Plate VII, fig. 28) where certain hyphae give rise by digitate branching to the palisade layer.

Entoloma

Collection and Preparation of Material.—Embedded material of three species was turned over to me by Professor Atkinson, who had collected the young stages of *E. flavifolium* and *E. grayanum* from rich leaf mold in the Michigan Hollow Swamp near Danby, N. Y., in September, 1914. The material of *E. cuspidatum* was gathered during July, 1916, from humus among sphagnum in the woods near Seventh Lake, N. Y. All of the material was fixed in the field in medium chromo-acetic acid. It was cleared in cedar oil and embedded in 52° paraffine. Sections were cut from 5 to 7 μ in diameter. Great difficulty was experienced in staining them. The young stages remained practically unstained in a great variety of the common stains. Finally the method which proved satisfactory for some of the resistant *Cortinarius* (15) species, that of using tannic acid as a mordant followed by the fuchsin stain, was tried with fairly good results. Iron-alum haematoxylin was very satisfactory for older stages.

E. flavifolium Pk.

(Figs. 82–100)

Early Stages.—The youngest button which was found is flask-shaped, about 3 mm. in length and 1 mm. through the widest part at the base (fig. 82). It is very compact in structure, especially in the region of the tip, where the hyphae are densely interlaced. The latter are slender and average about 2 μ in diameter. The many prominent nuclei are an indication that active growth is taking place here. Already this has resulted in the differentiation of an enlarged apex, the pileus primordium, from that of the stem. As one passes

to the base of the fruit body, where growth is less active, the tissue becomes more and more loose, the hyphae broad (6 μ), and almost unstained. Completely covering the young button is a thin, deeply stained layer, made up of large, thick-walled hyphae, in a more or less complete state of disorganization. This is evidently not a protoblem, such as is present in *Agaricus compestris* (3) and probably in certain species of *Cortinarius* (15), but appears to be formed as in the case of *Mycena subalcalina* by a transformation of the tissue on the outside of the fruit body by substances in the substratum, though which it has pushed its way. As the plant grows older, this outer stratum of dead elements is exfoliated.

The Primordia of Pileus, Stipe and Hymenophore.—When the fruit body has reached the stage of figure 83, a sharp constriction has formed below the free end by the branching and epinastic growth of the hyphae. As in the preceding species, this groove marks the division between the primordia of the stem and pileus. The whole fruit body has increased considerably in size, partly on account of the spreading out and loosening of the tissue and partly because of the addition of new elements. On the top of the pileus fundament the ends of the radial fibers break off in small mats or tufts, while just beneath the strongly curved margin and extending for a short distance down the stem is now distinguishable the primordium of the hymenophore (figs. 85, 86). This region, contrary to the condition in the preceding species, does not take the stain readily. It appears on closer examination to be composed of blunt hyphae with a somewhat irregular direction, but showing a marked tendency to turn downward perpendicularly to the pileus or outward from the stem.

The Palisade Layer.—These hymenophore elements increase very rapidly by branching and at the same time become considerably broader, so that a very compact palisade layer results (figs. 85, 86 and 98). It still is very resistant to the stain, but is easily distinguishable from the looser pileus and stem tissues adjacent to it.

The Development of the Gills.—The method of development of the gill salients is precisely the same as described in the preceding species in this paper and in the case of the previously mentioned endogenous forms. Figures 87–90 represent serial sections from the youngest fruit body which shows traces of these folds. They are protected in their development by the strongly inrolled pileus margin, which causes the apparent gill cavity in figures 89 and 90.

As soon as the young ridges begin to form, certain deeply staining elements make their appearance among the unstained ones of the original palisade (figs. 89 and 99). They grow rapidly and soon outstrip the others. Later on (figs. 91–95, 100) the unstained elements become completely lost amongst the deeply stained ones, which appear now to be young basidia. Is it not possible that the earlier unstained hyphae represent sterile paraphyses? A somewhat similar condition in *Cortinarius cinnamomeus* (15, Plate XI, fig. 48) suggests a like interpretation for the thin zone on the outside of the deeply stained hyphae. In the median section of this later series (fig. 91) we see the surface view of one of the primary gills. It is interesting to note that the palisade layer formed on the stem is developing into the decurrent tooth of the gill. Figures 92–95 represent longitudinal sections of the same fruit body. As one progresses outward, secondary salients are now making their appearance between the primary ones. Figure 100 is an enlargement of a portion of figure 94 and shows the young basidia pushing out from the surface of the gill just previous to the development of the sterigmata and spores. In figures 96 and 97 the fruit body is practically mature.

Entoloma grayanum Pk.

(Figs. 101–120)

Early Stages. The development of *E. grayanum* may be considered somewhat more briefly, inasmuch as it follows very closely the method of development described for *E. flavifolium*. The youngest button (fig. 101) has already become sufficiently well differentiated to make distinguishable the primordial regions of stem and pileus. On the surface there is also a layer of tissue which appears to result from the disorganization of the superficial cells, indicating an early stage in the retreat of pileus development from the surface to the interior of the young fruit body. The hyphae making up the fruit body are very compact and take the stain with the greatest difficulty. As the plant increases in size, its tissues become more open and the irregular primordium of the hymenophore makes its appearance in the groove formed by the arching out of the pileus (figs. 103–105). This very soon becomes organized into the even palisade layer shown in figures 106–110, which becomes very compact and remains inconspicuously stained (fig. 110) as in the former species.

Formation of the Gills.—Very early stages in the formation of the gills were not obtained, but the serial section (figs. 111–115) of an older fruit body, which is developing gill ridges, shows stages corresponding to those passed through in the development of *E. flavifolium* (figs. 87–95). The gills are, however, much more crowded than in that species. Certain elements on the surface of the folds in *E. grayanum* stain very deeply and become very conspicuous (figs. 112, 113, 119, 121). These are immature basidia, each of which contains at this stage a single large nucleus (fig. 121). They are not as numerous as they are in an earlier stage in *E. flavifolium*, where they formed a conspicuous stratum in the palisade layer (figs. 89, 99). It may be due to differences in the staining reactions. Figures 116–120 represent a slightly older stage of development. The gill salients are so crowded that they are developing somewhat irregularly. In figure 116 the decurrent tooth on the stem is noticeable.

ENTOLOMA CUSPIDATUM Pk.

(Figs. 122–139)

Early Stages.—Rhizomorphs of parallel hyphae produce the fruit bodies which are at first nearly homogeneous in their composition (fig. 122). By progressive growth at the apex there is formed a button in the stage of figure 123, which is just beginning to show a differentiation into pileus and stem primordia by the formation of an annular furrow. Later (figs. 124 and 125) the differentiation of these two main regions becomes more marked. The tissue in these fruit bodies was somewhat shrunken in the preparation processes, so that one cannot determine in them with certainty whether the primordium of the hymenophore has as yet developed. It can, however, be definitely ascertained in figures 126 and 127 and 138, where it consists of narrow, crowded, pointed elements, which take the stain readily in contrast to the looser subhymenial tissue. This soon gives place to an even palisade tissue (figs. 128, 129 and 137). Here the hyphae have increased in diameter, are very crowded and, as was noticed in the case of *Hygrophorus miniatus*, are more deeply stained next to the subadjacent tissue.

Further Development of the Fruit Body.—The gills appear as in the case of the two previous species as centrifugally growing ridges,

caused by the crowding of the palisade tissue and the pushing downward of the palisade layer by the elongation of the subadjacent tissue in regularly spaced intervals (figs. 130–134). As these folds develop, their surface becomes again uneven (fig. 139) as it did in case of the *Hygrophorus* forms mentioned above. Figure 135 represents a fruit body which is nearly mature. In figure 136 one may observe the spores from a mature plant. As in all the characteristic species of *Entoloma* the spores are angular. In this species they are very nearly cubical.

Summary

1. The fruit bodies of these exogenous forms come from buttons composed of interwoven hyphae, mainly extending in a longitudinal direction; with the exception of the surface layer, which is sometimes transformed by substances in the substratum through which the plants are growing, the tissue is entirely homogeneous.

2. Differentiation of the pileus and stipe primordia is brought about by growth at the apex of the fruit body. The hyphae multiply by profuse branching and begin to turn outward. This soon results in an enlarged pileus fundament, differentiated from that of the stem by an annular furrow. The tissues of both regions remain homogeneous in character throughout the life of the plant.

3. The fundament of the hymenophore is differentiated in the annular furrow. In the three *Hygrophorus* species, it seems to appear simultaneously with the differentiation of the pileus and stipe primordia, while in those of *Mycena subalcalina* and the three *Entoloma* ones, it develops slightly later. It is characterized by crowded, narrow, usually pointed hyphae and by an irregular surface.

4. Before the formation of gill salients an even palisade layer is usually formed by dense, broader hyphae with blunt ends. In two *Hygrophorus* species, *H. miniatus* and *H. nitidus*, however, the surface does not become even until after the gill ridges have formed.

5. The gills are formed from salients which appear first at the stem and develop in a centrifugal manner to the margin of the pileus. The dense crowding of the elements of the palisade layer results in a strong tension of the tissues, which is finally taken care of by the development of the subadjacent tissue downward into regularly spaced radial ridges. This method is precisely the same as that described by the early workers (**11**, **12**, **13**, **14**, **17**, **18**, **19**) and by Blizzard (**9**)

for other exogenous forms. Except for the fact that these gills develop on the exposed under surface of the pileus and not within a gill cavity, their method of origin is the same as that of the endogenous forms of the *Agaricus* type recently studied (**1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **15**, **20**, **21**).

In conclusion I wish to express my sincere appreciation to Professor Atkinson for his unfailing interest and helpfulness in the preparation of this paper.

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DESCRIPTION OF PLATES I–VII

Figures 19, 22, 23, 24, 27, 43, 44, 45, 47, 52, 66, 72, 75, 99, 100, 121, 135, 136, 138 and 139 were taken with a Bausch and Lomb compound microscope, fitted with a Zeiss 4 mm. ocular and a 3 mm. objective. For figs. 46 and 110 a combination of ocular 6 and objective 3, for fig. 82 a combination of a 4 mm. ocular and 16 mm. objective, and for fig. 98 an 18 mm. ocular and 16 mm. objective were used. The other figures were photographed by means of an extension camera and Zeiss lenses, figs. 64 and 65 with a 35 mm. objective, figs. 96 and 97 with a 50 mm. objective, and the others by means of a 16 mm. Spencer Lens Co. photographic objective.

PLATE I

Mycena subalcalina (figs. 1–24)

FIG. 1. × 36 diameters. Two young fruit bodies. The larger, by the epinastic growth of the hyphae at the apex, is becoming differentiated into stem and pileus primordia.

FIG. 2. × 36 diam. A slightly older stage, in which the pileus and stem primordia are well differentiated.

FIGS. 3, 4, 5. × 36 diam. Median and two tangential sections of a fruit body, showing the primordium of the hymenophore.

FIGS. 6 AND 7. × 36 diam. Median and tangential sections of a fruit body with the palisade layer developed. The crowding of the palisade elements causes the subadjacent tissue to become very loose.

FIGS. 8–12. × 36 diam. A series of sections from a fruit body, in which the gill salients are just beginning to form.

FIGS. 13–18. × 36 diam. A slightly older series, showing the development of the gills.

FIG. 19. × 255 diam. Enlargement of the margin of the section of fig. 2.

FIGS. 20 AND 21. × 36 diam. Median and tangential sections from a nearly mature fruit body.

FIG. 22. × 255 diam. Enlargement of the section of fig. 5, showing the hymenophore primordium.

FIG. 23. × 255 diam. Enlargement of the section of fig. 7, showing the palisade layer.

FIG. 24. × 234 diam. Enlargement of the section of fig. 16, showing young gills.

PLATE II

Hygrophorus miniatus (figs. 25–47)

FIG. 25. × 30 diam. Young fruit body before any differentiation has taken place. The deeply stained elements are nutritive hyphae.

FIG. 26. × 30 diam. An older stage, which shows the differentiation of stem and pileus primordia.

FIG. 27. × 192 diam. An enlargement of a fruit body, a little older than that of fig. 26, in which the primordium of the hymenophore is developing.

FIGS. 28 AND 29. × 30 diam. A median and tangential section of a fruit body just beginning to form gill salients. The palisade layer has not yet developed an even surface.

FIGS. 30–34. × 30 diam. A series of sections showing young gill salients.

FIGS. 35–39. × 30 diam. A series of sections of an older fruit body, showing the gills.

FIGS. 40–42. × 30 diam. A series of sections from a still older fruit body.

FIG. 43. × 209 diam. A portion of the surface of the pileus of the fruit body from which figs. 40–42 were taken.

FIG. 44. × 209 diam. An enlargement of the section shown in fig. 28, from a fruit body which is forming gill salients before a definite even palisade layer is formed.

FIG. 45. × 209 diam. An enlargement of the edge of a gill from the fruit body of fig. 41. An even palisade layer is now formed.

FIG. 46. × 566 diam. A further enlargement of the preceding.

FIG. 47. × 192 diam. An enlargement of the gill salients of the section shown in fig. 32.

PLATE III

Hygrophorus nitidus (figs. 48–66)

FIG. 48. × 31 diam. An early stage in which the pileus primordium is beginning to differentiate from that of the stem by the epinastic growth of the hyphae.

FIG. 49. × 31 diam. A slightly older stage in which stem, pileus and hymenophore fundaments are distinguishable.

FIGS. 50 AND 51. × 31 diam. An older stage, showing the primordium of the hymenophore further developed.

FIG. 52. × 210 diam. A higher magnification of the section of fig. 50 showing the hymenophore primordium.

FIGS. 53–58. × 31 diam. A series of sections from a fruit body in which the gill salients are just beginning to form. The subadjacent tissue has become very loose.

FIGS. 59–63. × 31 diam. An older fruit body, showing the development of the gills. Notice the very loose character of the subadjacent layer and the very irregular gill surface. The deeply stained hyphae on the surface of the pileus make up the slime-producing layer.

FIGS. 64 AND 65. × 12 diam. Median and tangential sections of a more mature fruit body. The plant is becoming umbilicate and the gills triangular in shape.

FIG. 66. × 210 diam. An enlargement of the section from which fig. 61 was taken, showing the uneven surface of the gills.

PLATE IV

Hygrophorus borealis (figs. 67–81)

FIG. 67. × 28 diam. Upper part of a young, oval, homogeneous fruit body.

FIGS. 68 AND 69. × 28 diam. Later stages. The buttons become very elongate.

FIGS. 70 AND 71. × 28 diam. The primordia of stipe, pileus and hymenophore have become differentiated.

FIG. 72. × 190 diam. An enlarged photograph of the margin of the pileus of the section of fig. 70.

FIGS. 73 AND 74. × 28 diam. Sections from a fruit body, showing the palisade layer.

FIG. 75. × 190 diam. The palisade layer of fig. 74 enlarged.

FIGS. 76–80. × 28 diam. A fruit body showing well-developed gill salients. The light area, which extends into the gills and between them in the pileus region subadjacent to the hymenophore, is the region which has given rise by branching to the elements of the hymenophore.

FIG. 81. × 28 diam. A nearly median section, with a portion of the gill attached to the stem.

PLATE V.

Entoloma flavifolium (figs. 82–100)

FIG. 82. × 35 diam. Young button in which the pileus primordium is beginning to develop.

FIGS. 83 AND 84. × 30 diam. A slightly older fruit body in which pileus, stem and hymenophore primordia have developed.

FIGS. 85 AND 86. × 28 diam. The palisade layer is distinguishable, although it does not take the stain readily.

FIGS. 87–90. × 28 diam. Serial sections from a fruit body in which the young gill salients are beginning to form.

FIGS. 91–95. × 28 diam. A series of sections from a more mature fruit body. Young basidia over the surface of the gills take the stain very readily.

FIGS. 96 AND 97. × 9 diam. Median and tangential sections of a nearly mature fruit body.

FIG. 98. × 160 diam. An enlargement of a section of a fruit body in about the stage of fig. 85, showing the palisade layer.

FIG. 99. × 191 diam. An enlargement of the section of fig. 89, showing very young gill ridges. Certain elements in the palisade layer are beginning to take the stain very readily.

FIG. 100. × 191 diam. An enlargement of the section of fig. 94, showing young basidia developing over the surface of the gills.

PLATE VI

Entoloma grayanum (figs. 101–121)

FIG. 101. × 33 diam. Young fruit body in which the pileus primordium is beginning to differentiate from that of the stem.

FIG. 102. × 33 diam. A slightly older stage.

FIG. 103. × 31 diam. Pileus and stem primordia have developed and the hymenophore fundament is commencing to form in the furrow between them.

FIGS. 104 AND 105. × 31 diam Median and tangential sections of a fruit body showing the primordium better developed.

FIGS. 106–109. × 31 diam. A series of sections, showing the palisade layer, just previous to the formation of the gill salients.

FIG. 110. × 518 diam. Enlargement of the section of fig. 108, showing the palisade layer.

FIGS. 111–115. × 30 diam. A series of sections, forming young gill ridges.

FIGS. 116–120. × 30 diam. Slightly older stage, showing the same. The black dots appearing on the margin in fig. 119 are young basidia.

FIG. 121. × 191 diam. An enlargement of the gill salients of the section of fig. 119. The nuclei of the young basidia are very prominent.

PLATE VII

Entoloma cuspidatum (figs. 122-139)

FIG 122. × 32 diam. Young undifferentiated fruit body, still attached to a rhizomorph.

FIG. 123. × 32 diam. The fruit body by growth at the apex is beginning to differentiate into the pileus primordium.

FIGS. 124 AND 125. × 32 diam. Older stages showing the pileus and stipe primordia.

FIGS. 126 AND 127. × 32 diam. Median and tangential sections of a fruit body, in which the primordium of the hymenophore has made its appearance.

FIGS. 128 AND 129. × 32 diam. Median and tangential sections from a fruit body, which has developed the palisade layer.

FIGS. 130–134. × 32 diam. A series of sections, showing the development of young gill salients.

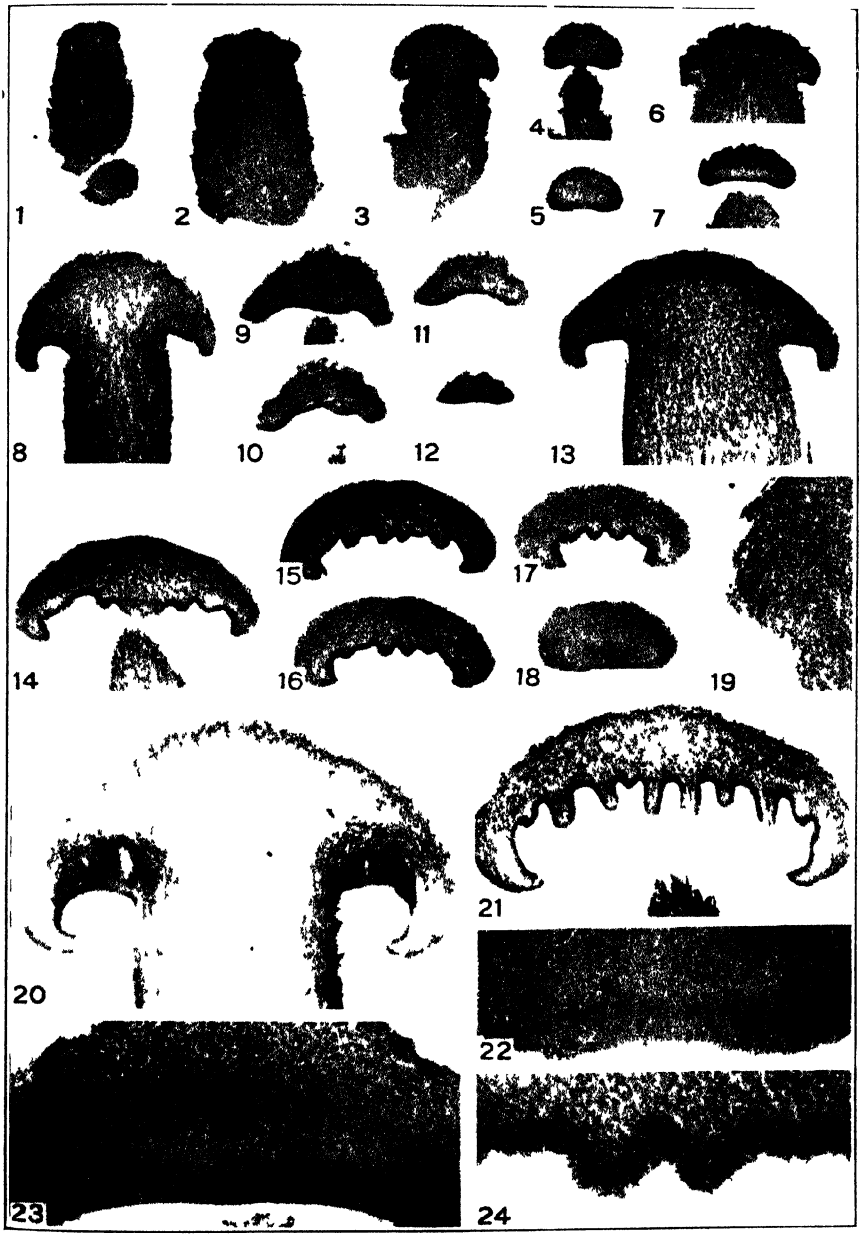
FIG. 135. × 29 diam. Median section of a nearly mature fruit body.

FIG. 136. × 213 diam. Section of the gill of a mature plant, showing the cubical basidiospores.

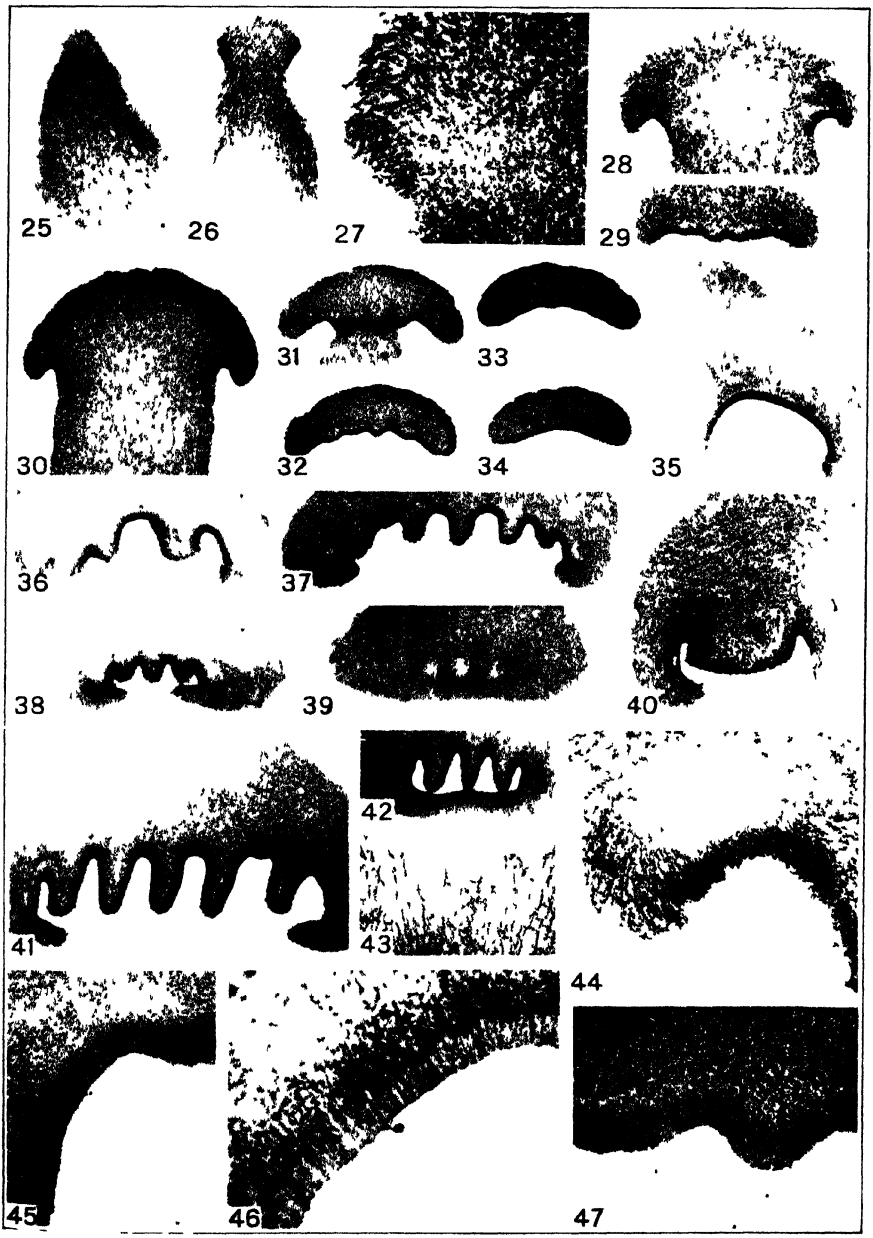
FIG. 137. × 213 diam An enlargement of the section of fig. 128, showing the palisade layer.

FIG. 138. × 213 diam. An enlargement of the section of fig. 126, showing the hymenophore in a primordial state.

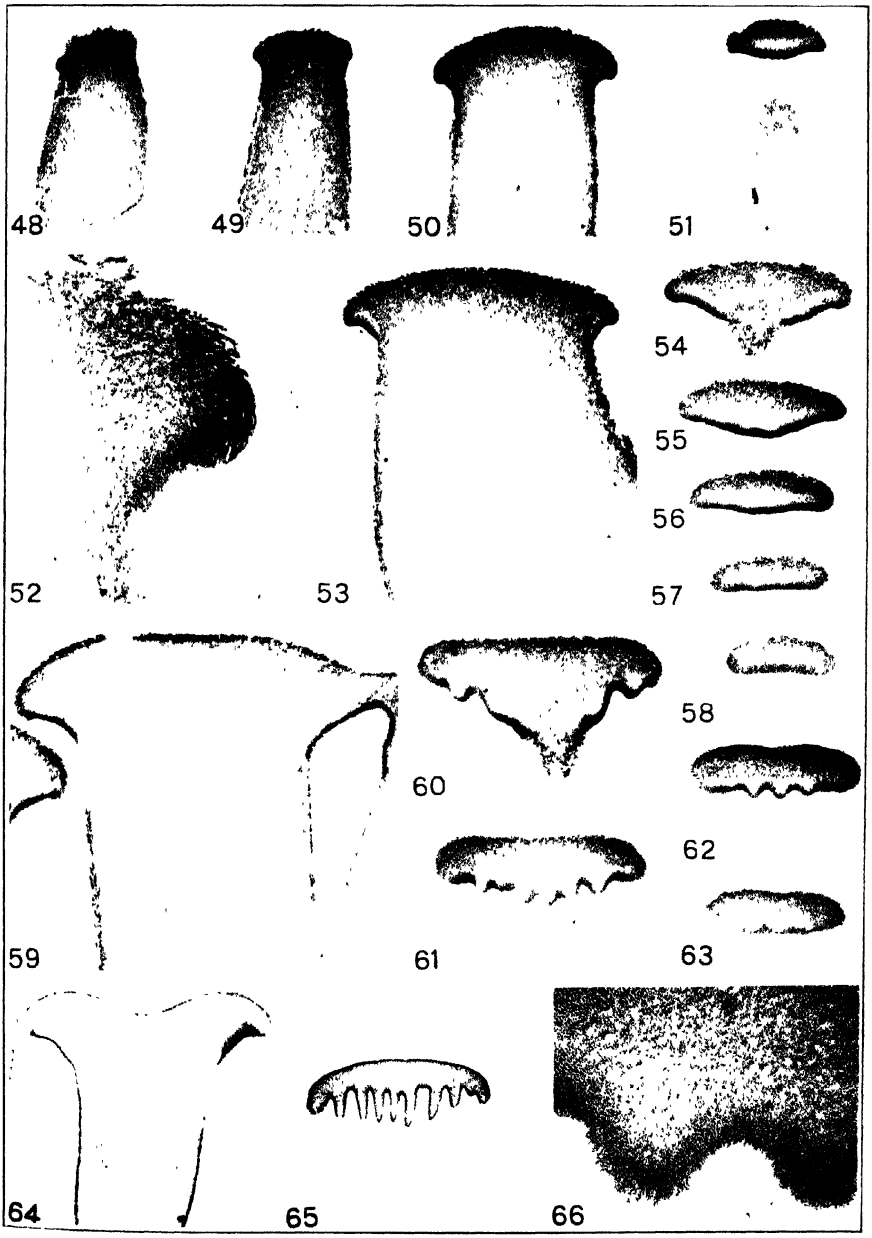
FIG. 139. × 213 diam. An enlargement of the section of fig. 134, showing the young gills.



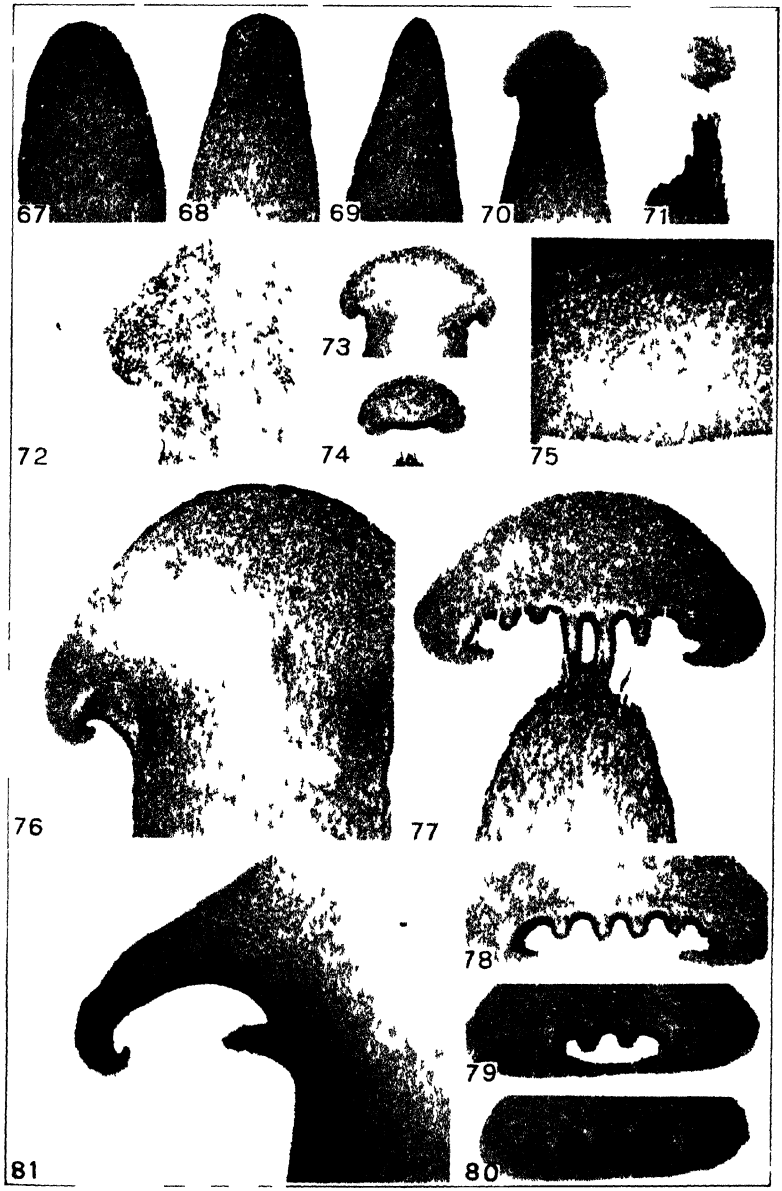
DOUGLAS ? MYCENA SUBALCALINA



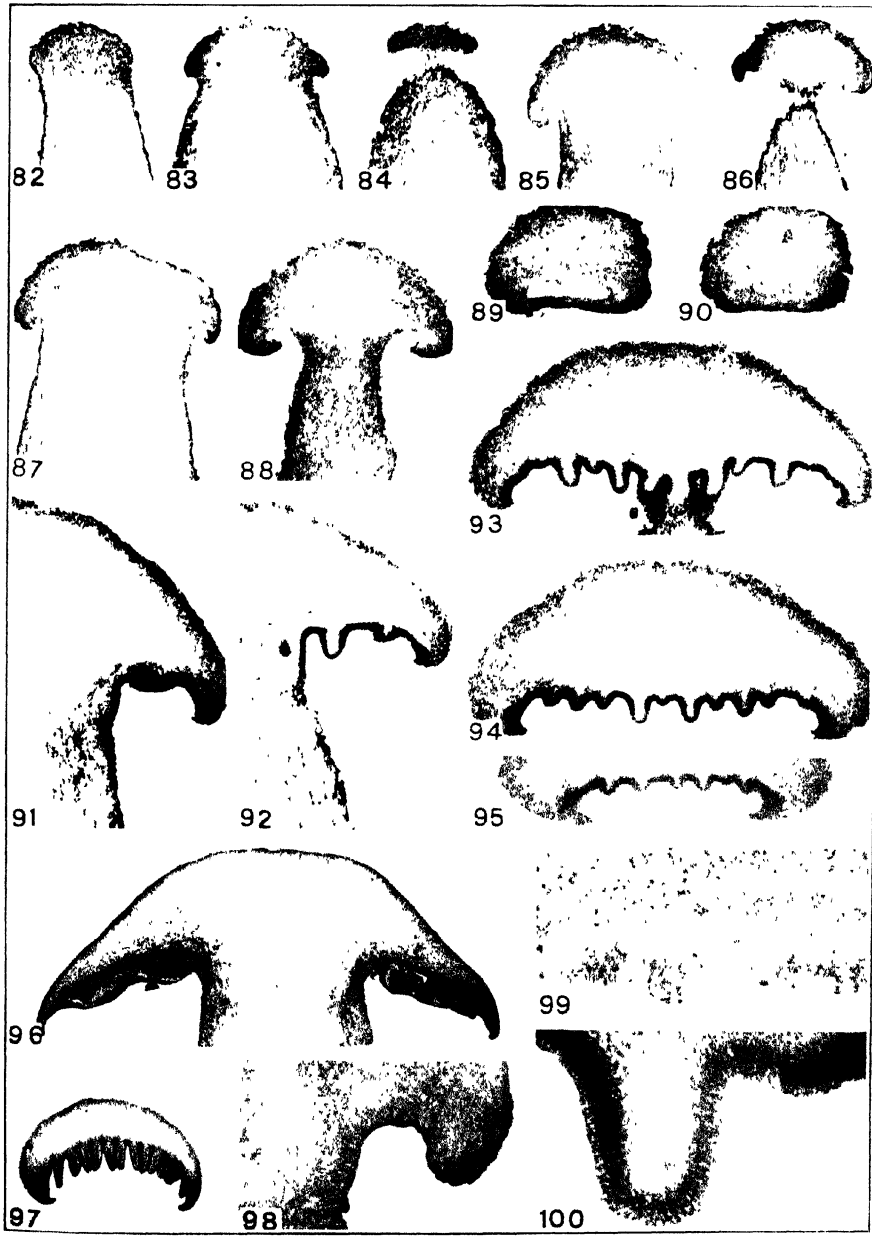
DOUGLAS HYDROPHORUS MINIATUS.



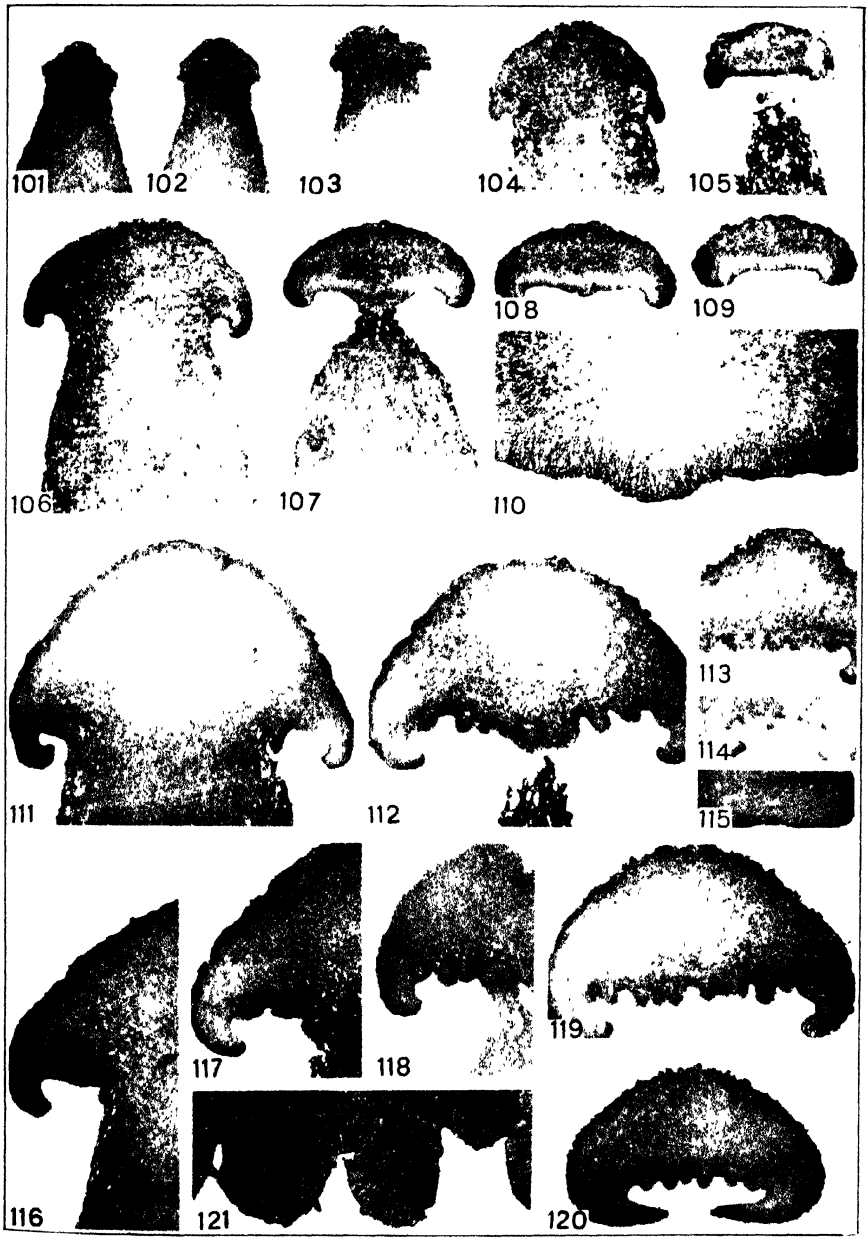
DOUGLAS: HYGROPHORUS NITIDUS.



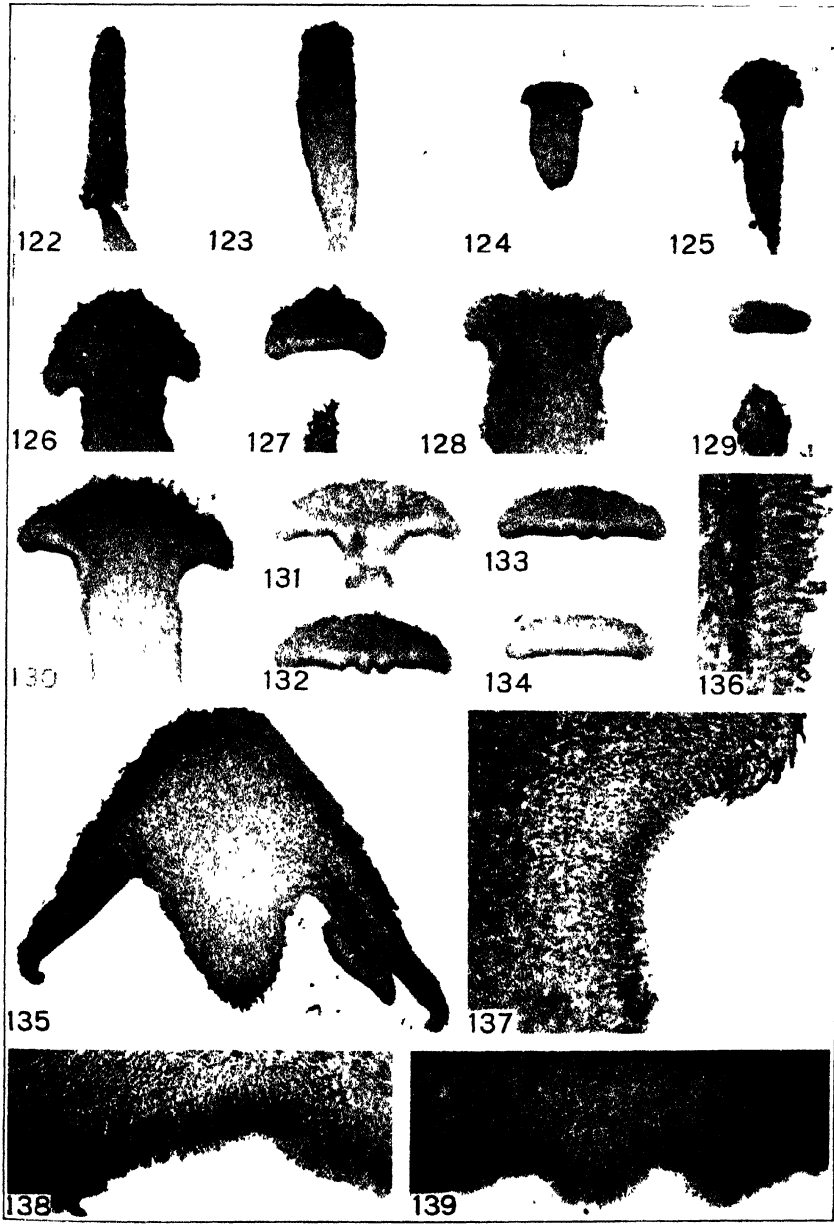
DOUGLAS: HYGROPHORUS BOREALIS



DOUGLAS. ENTOLOMA FLAVIFOLIUM.



DOUGLAS: ENTOLOMA GRAYANUM.



DOUGLAS: ENTOLOMA CUSPIDATUM

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THE STRUCTURE OF THE UREDINIUM IN PUCCINIASTRUM AGRIMONIAE

C. A. LUDWIG AND C. C. RITS

A number of genera of the Melampsoraceae (Uredinaceae) are characterized in the uredinial stage by definite, punctate, usually small sori. Each sorus is surrounded by a peridium composed of cells which are more or less isodiametric when seen in face view and which form a membrane-like tissue. The genera Pucciniastrum, Melampsoridium, and Melampsorella, belonging to the subfamily Pucciniastratae, have uredinia of this type. Although the peridium is quite fragile in appearance, it is nevertheless true that the sorus maintains its shape remarkably well; and the spores make their escape only by a central pore until the sorus is quite aged. The spores, whether borne in chains or on pedicels, are easily loosened from their attachment and so, quite early in the development of the sorus, lose their original arrangement and become packed in without any special order.

The customary method of studying rust morphology with dried herbarium material (*i. e.*, by scraping up the spores or by cutting free-hand sections) is not sufficient, in the case of many sori of this sort, to give results of reasonable certainty. Especially is this true when an attempt is made to learn the manner in which the spores are borne, although such technique is amply satisfactory for the study of coarser details in many other kinds of sori. It has been only rather recently, however, that a full realization of the limitations of the value of free-hand sections in this group has come about.

The mature urediniospore (fig. 4) has an echinulate wall and usually an easily discernible hilum, both of which features are especially characteristic of pedicellate spores. When, therefore, such spores were

found without any special arrangement in the sorus the situation was not considered unusual. This condition is common with uredinia and, coupled with a marked indistinctness of the structures at the base of the sorus, led naturally to the conclusion that the spores were pedicellate and that the short pedicels were concealed in what appeared to be a mass of intertwining hyphae. Under this impression the descriptions and classification of the Pucciniastratae were published by Arthur[1] in the North American Flora, work previous to that time having thrown no doubt on the correctness of the view.

The first intimation that the accepted idea might be wrong came with the announcement by Liro[2] that the urediniospores of *Melampsorella Cerastii* (Pers.) Wint. are borne in chains. This point was developed somewhat and the sorus of *M. Cerastii* figured the next year in a paper by Magnus.[3] The work of these two investigators, therefore, raised the question as to whether or not the urediniospores in the genera with similar sori were not also catenulate. It became, consequently, a matter of some importance to study these rusts in a more careful way than is possible with free-hand sections.

The writers began their work on October 3, 1913, by fixing some sori of *P. Agrimoniae* from leaves of *Agrimonia parviflora* Sol. The material was found growing near Lafayette, Indiana, and was the only fresh material of the group available. Part of it was fixed in chromoacetic acid and part in Flemming's weaker solution. It was imbedded in paraffin in the usual way and stained with the triple stain, the chief aim being to study the morphology of the sorus rather than nuclear phenomena.

From the material in hand it has been possible to make out that in *Pucciniastrum Agrimoniae* (Schw.) Tranz. the uredinium begins as a small aggregation of hyphae under the epidermis. Presently some of the hyphae become erect, thickened, and divided by cross walls into three or four cells each. The apical cells of the columns elongate considerably and the protoplasmic contents become less dense, as is shown by a tendency to stain less deeply than at first. They are evidently the first peridial cells to be differentiated and the other cells of the chains are spores. No intercalary cells were seen and no chains were observed having more than three or four spores to

[1] N. Amer. Fl. 7: 97, 105–117. 1907.

[2] Uredineae Fennicae Finlands Rostsvampar 490, 492. 1908.

[3] Ber. Deutsch. Bot. Ges. 27: 320–327. 1909.

the chain. The peridial cells at this stage are much longer in a vertical direction than they are in the mature sorus, and their side walls, instead of being oblique, are perpendicular (fig. 1). The later change in shape is probably due to the pressure of the spores as they are produced. Such a pressure coupled with the decreasing cell turgor would tend to collapse the cells somewhat and to press their bases laterally.

Sections at a later stage show that as the sorus develops the epidermis of the host is lifted from the mesophyll; and this separation of the surface layer from the underlying tissues often extends for some distance beyond the very definite lenticular sorus. The mesophyll tissues are apparently not at all or only slightly injured. Often the cells directly under the center of a mature sorus retain their shape perfectly. Only a little mycelium can be seen under the sorus and none beside or over it; in fact the amount of vegetative mycelium visible at any stage is very small. The mature sorus (fig. 3), as may be inferred from what has just been said, is a very clearly differentiated structure which is sharply set off from the host tissue and from the vegetative mycelium. It is bounded above and at the sides by a peridium of somewhat overlapping, thin-walled cells in which the overlapping above the center is in a manner opposite to that of the shingles on a roof. At the side of the sorus, the direction of the overlapping becomes indefinite, so that from this point to the hymenial layer the cross walls may be oblique in either direction or some in one and some in another. The cells are usually so much collapsed that it is impossible to make out details accurately even in stained paraffin sections; but in spite of its fragile appearance it is evident that the peridium has considerable tensile strength, for the sorus maintains its shape and, until it is quite aged, furnishes no means of escape for the spores except a central ostiole. At this point, the peridium later begins to disintegrate. The ostiole is bordered by a ring of cells (fig. 2) which are larger and thicker walled than those of the rest of the peridium.

The base of the sorus is characterized by a plate-like hymenial layer of hyphal cells which at its margin often appears almost or quite continuous with the peridium and which separates the sorus from the underlying structures with a definiteness unusual in rust sori. The spore chains rise from a layer of basal cells just above the hyphal plate layer. In the young sori, where the chains are clearly visible,

of more nearly approximating a natural order. It has now been shown the plate-like layer is not very evident and in mature sori the chains are difficult to make out.

The spores and other cells which go to make up the uredinia, and the telia as well, are binucleate. Such a condition is naturally expected when the sporophytic stage of a pleomorphic rust is under consideration. The nuclear state of the free hyphae could not, however, be determined; but doubtless they are also binucleate. Nothing in the nature of hyphal fusions was observed. This is not unexpected in the consideration of the uredinial habits of this species, for normally fusions occur in the aecial stage; and all species of the genus Pucciniastrum are presumably heteroecious.

The mature urediniospores (fig. 4) of *Pucciniastrum Agrimoniae* are obovoid in shape, not angular, and, as already mentioned, have a more or less distinct hilum. The wall is distinctly echinulate. These are characters commonly associated with pedicellate spores, and when found in catenulate spores, where only short chains can be seen, must evidently be taken to indicate that the terminal spore matures and becomes detached before the next one has advanced far in its development. The spore next to the free end of the chain then develops in exactly the same manner, and so the process is repeated as long as spore production continues. The association of the catenulate habit and the echinulate condition is worthy of remark. The writers are unfamiliar with any other genus, except Melampsorella, in which such association has been shown, although presumptively it may also occur in the nearly related Melampsoridium. Catenulate spores are usually verrucose except in the case of some teliospores, in which instance they are smooth, never echinulate.

The peridium, as mentioned above, is formed in a way analogous to that in the ordinary aecidium. One wonders how it is produced in similar sori with pedicellate spores, as in the fern genera Uredinopsis, Hyalopsora, and Milesia, and could wish that Bartholomew's[4] recent work with *Hyalopsora Polypodii* had cleared up the point for that species. It would perhaps not be too daring to risk the opinion that in such cases the roof cells are formed from the first urediniospores and produced in the same manner as in sori in which the spores are borne in chains.

With the data now at hand it would seem to be possible to suggest a new arrangement of the genera of the Pucciniastratae with some hope

[4] Bull. Torrey Bot. Club 43: 195–199. 1916.

that the urediniospores in certain species of Melampsorella and Pucciniastrum are catenulate, although sometimes appearing much as if pedicellate; and it does not seem to be unreasonable to anticipate that the same condition is to be found in Melampsoridium. In addition, it is now also definitely established by Bartholomew's work that the true pedicellate habit is to be found in Hyalopsora; and it is at least highly probable that the same condition is typical of the other fern rusts. Now, if these assumptions prove correct, there is provided a set of invariable characters upon which the larger divisions of the group may be separated. The points of distinction formerly used, such as wall color or number of cells in the teliospore, are often variable to a large degree within the same sorus.

This method of dividing up the group would give two subgroups, in one of which the urediniospores are borne singly on pedicels and in the other of which, when present, they are borne in chains with each chain maturing but one spore at a time. To the first of these two subgroups would belong the three fern rust genera Uredinopsis, Milesia, and Hyalopsora; while to the latter would belong the genera Pucciniastrum (Thekopsora), Melampsorella, Melampsoridium, and Calyptospora. Calyptŏspora, a genus without uredinia, is nevertheless included because such characters as it possesses show close affinity to Pucciniastrum. This genus, therefore, gives no real trouble in this connection, even though it could not be taken care of in a working key if uredinial characters only were used in the division.

The division of the Pucciniastratae along the line suggested has the additional fact in its favor that it groups the fern rusts together and separates them from those rusts of the group which have the sporophytic stage on spermatophytes. Because of the evident similarity of the fern rusts, such a change has always been desirable, but until now lack of sufficient morphological information has prevented it.

It is possible, of course, that further study within the group will bring to light facts which will make necessary a further realignment of the genera. However, from the data now at hand it would seem that the grouping here suggested is more nearly along natural lines than any heretofore proposed.

The writers wish to acknowledge here the aid received from Dr. J. C. Arthur, Prof. H. S. Jackson, and Dr. F. D. Fromme, to whom they are indebted for a number of helpful suggestions.

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EXPLANATION OF PLATE VIII

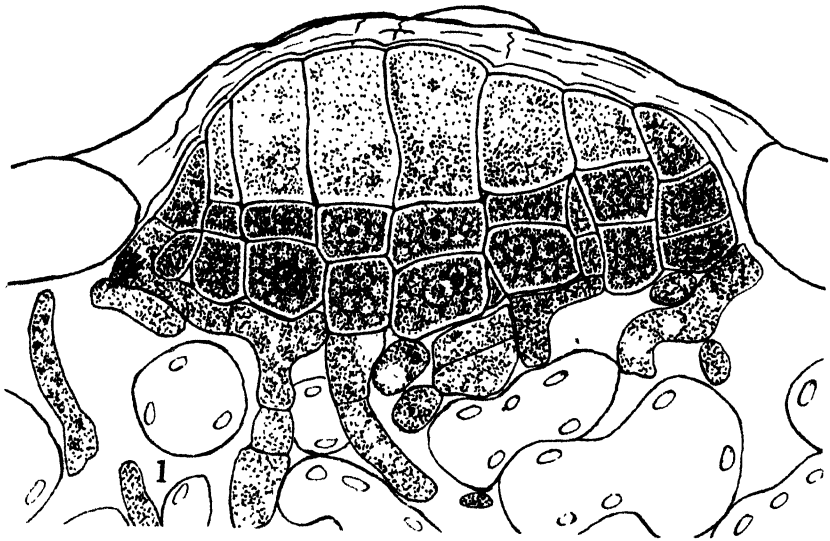
All drawings were made with the camera lucida, and all illustrations are from stained microtome sections except No. 4, which was made from spores scraped from a dry herbarium specimen and mounted in water.

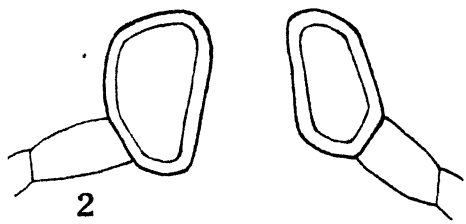
FIG. 1. Young sorus of *Pucciniastrum Agrimoniae*. × 1360.

FIG. 2. Vertical section of ostiole of mature sorus. × 1360.

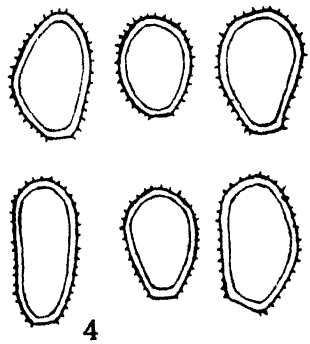
FIG. 3. Mature sorus of *Pucciniastrum Agrimoniae*. × 290.

FIG. 4. Outline sketch of urediniospores. The hilum is indicated at the bottom of the spore in each case. × 825.









LUDWIG AND REES: UREDINIUM OF PUCCINIASTRUM.

SPORE FORMATION IN PHILOCOPRA COERULEOTECTA

HALLY JOLIVETTE SAX

The greater part of the work done on spore formation in the Ascomycetes has been carried on in connection with the few-spored asci. The many-spored forms have received little attention due to the difficulties attending the smaller size of the spores in most cases. The process of delimitation of spores in a many-spored ascus is of special interest in that it may throw some light on the true nature of the ascus. The variations from the method found in the few-spored forms call for further study.

The review by Sands (24) of the literature on the process of spore delimitation in the Ascomycetes, as well as the monograph by Guilliermond (11) on the cytology of the Fungi, makes it unnecessary to give a detailed account of the work done. A brief review will suffice to show the present status of the question as well as the problems at hand.

The process of spore delimitation in the Ascomycetes was first described by Harper (14). He found that the spores were cut out of the cytoplasm by the bending back of the astral rays and their subsequent fusion to form an ellipsoidal membrane. Harper (12, 13, 14, 15, 16, 17) has described the process in a number of different genera. His work has been corroborated by work on additional forms by Guilliermond (8), Maire (21), Sands (24), the writer (18), and others. This view is opposed by Faull (1) who believes that the membrane is formed at the expense of a granular zone of cytoplasm. He believes the astral rays play no part in the delimitation of the spores.

Harper's view that the astral rays are of the nature of cilia is opposed by Fraser (3, 4), who agrees with him that the spores are delimited by the astral rays in the forms studied by her. Following the hypothesis suggested by Harper (14, page 274)—that the centrosome may be the seat of fermentative activity—Fraser holds the view that at the close of the third division the centrosome may generate a ferment, which, as the nucleus pushes out toward the wall, flows back in its wake, producing a chemical change in the area through which

it is distributed. However, there can be no question from the figures described for Geoglossum by the writer (18) that the rays actually bend down toward the nucleus in order to form the membrane. The long duration of the period when the interastral zone is formed by the rays, which run out radially from the center in all directions, renders Fraser's theory quite untenable.

Overton (22) has described spore formation in *Thecotheus pelletieri*, a thirty-two-spored form. He found it to be similar in general to that described by Harper for the few-spored forms. In *Thecotheus* thirty-two nuclei were formed before delimitation. Ramlow (23) reports a similar method of spore formation in *Thelebolus*. The spores are delimited after many nuclei are formed.

Lewis (19) studied the development of the ascus in *Pleurago zygospora*, a sixteen-spored form. The nuclei in this case undergo the usual three divisions, producing eight nuclei, after which delimitation occurs. The spore then elongates to form a filament which subsequently divides to form two spores.

While carrying on some experiments on the light reactions of *Pilobolus crystallinus* at the University of Wisconsin, it was noted that after a culture had been in use for several days, there appeared on the glass used in the experiment some black masses much smaller than the sporangium of Pilobolus. A further examination revealed the fact that these spots contained the spores from a Pyrenomycete. At first this fungus covered the surface with delicate hyphae, which increased until the culture was coated with a white mat of mycelia. Gradually there developed from this mycelium many black flask-shaped fruiting bodies. At first these appeared as little black specks on the white mycelium, but later they covered it in great numbers. The Pilobolus died out as this Pyrenomycete grew. In connection with the work on Pilobolus, which demanded that the glass on which the spores were found be placed at varying distances from the culture, it was noted that this fungus, as well as Pilobolus, could throw its spores a considerable distance and that they were discharged toward an illuminated spot.

The asci from the pyrenocarp contain many spores. It was found that they did not agree with the description given for any known species. The material was sent to Dr. Rehm for identification. Rehm described it as a new species and gave it the name *Philocopra coeruleotecta* Rehm.

The asci were of fairly good size and appeared favorable for the study of their development and the formation of the ascospores in a many-spored form. The many-spored forms are looked to for evidence on the question whether the ascus is in any way related to the sporangium found in the Phycomycetes. The process of delimitation in a many-spored ascus; the stage when delimitation occurs; the presence of a central body; and the relation of the epiplasm and spore-plasm are subjects of considerable interest in the study of the relationships of the Ascomycetes.

The material for this study was fixed in Flemming's weaker solution and Strasburger's modification of Flemming's medium. Both afforded excellent fixation. The sections were stained with modifications of Flemming's triple stain and Heidenhain's iron-haematoxylin.

There was no trouble in getting an abundance of the material in all stages of development. The fruiting bodies, together with the adjacent mycelium, were fixed in large quantities. The substratum was included in many cases in order to get all stages. It proved to be very easily sectioned. There was considerable variation in the stages of development among the different asci in a pyrenocarp. Sometimes mature spores and very young asci were found in the same fruiting body. There were always at least several stages to be found until the pyrenocarp reached its full size.

The pyrenocarp is flask-shaped. The wall is composed of several layers of closely packed hyphae, the walls of which stain darkly. From its base arise a number of asci among which are seen numerous paraphyses. Often the asci in a single pyrenocarp are in various stages of development. There is considerable variation in the number of asci to be seen in a pyrenocarp, depending somewhat on its stage of development. Figure 1 represents a longitudinal section through a pyrenocarp. It shows the typical flask shape with the opening in the neck at the apex. This section shows three immature asci with numerous ascospores. Often as many as eight or ten asci may be seen in a single pyrenocarp with the spores at different stages, affording a good opportunity to trace the progressive steps in their development. Younger ascocarps are, however, more favorable for the study of the young stages before spore formation begins.

In figure 2 is shown a very young ascus. It was located very near the base of the pyrenocarp. The rounded upper portion contains

one large nucleus with a distinct nucleole. There is present a clear staining central body, which in this case is opposite the nucleole. The chromatin is distributed in a fine network and shows more or less orientation with regard to the central body, *i. e.*, the chromatin granules run inward from the center in more or less distinct rows. The cytoplasm is comparatively dense at this stage. This stage is only of short duration as the ascus elongates rapidly, taking the more slender form seen in figure 3.

Figure 3 represents a young ascus, which is very slender and cylindrical in shape. At this time the nucleus is usually situated in the central portion of the ascus, in this case being nearer the lower end. The cytoplasm is rather uniformly distributed and appears to have a fine granular structure. A few radiations in the cytoplasm extend outward from the nucleus. The nucleole is not visible in this section. The chromatin is in fairly large masses for the most part, and some connection is visible between those particles.

The ascus undergoes a rapid increase in size, so that when the first division of the nucleus occurs it appears to have become several times its original size. The first division of the primary nucleus is shown in figure 4. The cytoplasmic mass is very great compared with that of the nucleus. The cytoplasm in the upper part of the ascus is very similar to that shown in figure 3. The cytoplasm of the lower portion appears a little more vacuolate. The spindle figure is somewhat curved and very distinct and has a small but clearly staining central body at each end. The chromosomes are few in number. They are short and stain heavily. Fibers extend out from the centers but they are not at all distinct between the plasma membrane of the ascus and the centers in this figure. However, there is a very noticeable bulging out of the membrane opposite the central bodies which may indicate some relation between the membrane and the division figure, such as the radial arrangement of astral rays extending outward from the central bodies. Fibers may be seen extending upward and downward from the central bodies. Those below the spindle were especially distinct and in the plane at which figure 4 was drawn they appear to converge at a considerable distance from the central bodies, making the astral rays appear considerably longer than those of the spindle. The division figure lies crosswise of the ascus.

The nuclear divisions in the ascus follow one another rapidly. The daughter nuclei divide while still in close proximity to each other.

Thus the nuclei occupy the part of the ascus nearest the place where the division of the primary nucleus occurred. The daughter nuclei in each case are completely formed before entering upon a new division. The spindle is intranuclear at first. Often the nuclear cavity remains distinct until the early anaphase.

Figure 5 shows two of the resting nuclei in the four-nucleated stage of the ascus. In this case the ascus was cut obliquely. The nuclei are fairly large and show much the same structure as was shown in the primary nucleus of the ascus. The nucleole and central body are distinct. The chromatin network is typical of that formed in the few-spored Ascomycetes and shows some orientation with respect to the central body. It has a lighter and a darker staining portion, the darker part forming lumps scattered along the finer light-staining thread-like portion. The cytoplasm is somewhat denser near the nuclei but there is no well-marked spore-plasm.

Figure 6 represents a dividing nucleus of the third division. One complete figure is shown which runs almost parallel to the length of the ascus. The spindle is slender. The chromosomes have divided and are passing toward their respective poles, where there is a small distinctly staining central body. The nuclear cavity is still apparent. There is here an interesting arrangement of the cytoplasm with regard to the central bodies. It is very dense in the immediate neighborhood of the centers, from which distinct radiations extend outward in all directions. To the right of the lower central body there is visible an oblique section through the polar end of a second spindle figure. The polar aster is apparent. The cytoplasm is fine with radiations running outward in all directions. They are very fine and dense in the immediate neighborhood of the central body.

In the eight-nucleate stage (fig. 7) the ascus has grown somewhat larger. The nuclei are close together in the region near where the primary nucleus was located. The cytoplasm is denser immediately surrounding the nuclei and more vacuolate in that further remote. The less uniform appearance of the cytoplasm is conspicuous in comparison with the earlier stages. The nuclei appear to be in a normal resting condition. They are much smaller than the primary nucleus. Each nucleus contains a distinct nucleolus. The chromatin appears in fine granules. The central body is visible in the best stained specimens, but is not always apparent. Only three of the eight nuclei are visible in the section.

Figure 8 represents a section of the ascus containing one spindle figure of the fourth nuclear division in the ascus. It is typical of all of the spindle figures present. The spindle with the chromosomes in the center stains densely. The nuclear cavity is still distinct. The central bodies at the poles of the spindle stand out clearly. Numerous delicate fibers radiate out from the central body. These are very long and remarkably distinct which is characteristic of the well-stained figures. The fibers, however, cannot be seen as such except when they lie in the plane of the section. They are extremely delicate, but the large numbers seen in the plane of this figure render them rather conspicuous.

In the sixteen-nucleate stage the nuclei in their successive divisions have gradually scattered through a larger part of the ascus, with the upper and lower portions, however, still containing only cytoplasm. The region of dense cytoplasm surrounding the nuclei is even more distinct than in the earlier divisions. From these denser regions strands of cytoplasm radiate outward in different directions. Figure 9 represents a longitudinal section of the ascus at the sixteen-nucleate stage. Eight nuclei are visible in the section. At this stage the nuclei appear much the same as after the previous divisions, except that they are smaller. The nuclei do not increase much in size after dividing. Their structure is very similar and they are not beaked.

The nuclei continue to divide rapidly until there are approximately 128 in number. The later divisions are essentially like the earlier ones. The spindles are small and with distinct centers at each pole. The astral rays, which are strongly developed, radiate out from the centers. These divisions take place near the periphery of the ascus. Figure 10 represents a portion of the ascus containing a nucleus in the equatorial plate stage. This is one of the late divisions. The spindle is very small but clear, with a distinct central body at each end. The chromosomes are plainly differentiated in the center of the spindle. The nuclear cavity is still apparent. The clearness with which this figure shows up in so small a nucleus is remarkable. The asters are present at the poles of the spindle. The astral rays can be seen extending outward from the central bodies into the cytoplasm. The fibers are extremely delicate, but apparent. The dense fine granular appearance of the remaining cytoplasm near the central body is probably due to the fact that numerous fibers have been cut in sectioning.

At the close of the last division the nuclei are situated near the wall of the ascus. They are very small but distinctly beaked. The central body is at the tip of the beak and appears to be in contact with the plasma membrane of the ascus. The nucleole may be situated at the end of the nucleus farthest from the central body, near the base of the beak, or on any one side. The chromatin is fine and granular. There is a dense region of cytoplasm around the nucleus. The cytoplasm in the center of the ascus is very vacuolate and apparently devoid of nuclei. The nuclei are conspicuously located near the wall. Figure 11 represents a longitudinal section through an ascus at this stage. The central part contains many vacuoles. The nuclei are peripherally located and surrounded by a dense cytoplasm. The beaks are visible on only four of the nuclei figured. The others have been cut across below the beaks in sectioning.

Figure 12 represents a cross-section of an ascus at the same stage. The peripheral arrangement of the beaked nuclei is even more manifest in this view. The large internal portion and the basal and apical regions of the ascus contain only cytoplasm. The centers are distinct and the nuclei are conspicuously beaked. The nuclei are very small at this stage.

Figure 13 represents a slightly later stage. The section has been cut somewhat obliquely from the side of an ascus. It shows only a small part of the ascus near the periphery. The nuclei with their long slender beaks are always pointing toward the membrane, the centers apparently in contact with the latter, although the nuclei in this stage, as well as in that represented by figures 11 and 12, always have the centers in contact with the plasma membrane of the ascus. They are comparatively small at this stage, but the fact that so many of them lie with their longitudinal axis in the plane of the section allows them to be easily observed. There is no definite arrangement as regards their exact direction. This is especially clear from figure 13. The fine cytoplasmic masses surrounding the nuclei are very conspicuous in this figure. Often little fibers can be seen radiating from the centers. A close study of the two beaked nuclei on the left of the figure shows the centers against the plasma membrane with an indentation of the membrane between as if the astral rays of the two nuclei meet in this region. On the other sides of these nuclei there is a similar appearance, reminding one of the stages in *Geoglossum glabrum* Pers. when the interastral zones are formed by the meeting

of the fibers of adjacent asters. The lower portion of figure 13 is nearer the periphery. Three or four dense masses of cytoplasm have been cut across. They appear rather closely approximated when seen from this view. The appearance here in general is characteristic of the stage when the interastral region is formed by the meeting of the rays from the adjacent centers prior to the delimitation of the spores. This stage is frequently met with and must be of comparatively long duration.

The spores are delimited while the nuclear beaks point toward the membrane. At the time of delimitation the spore is practically spherical in shape. The membrane is very delicate at first. The cytoplasm contained within the spores is finely granular and dense. The epiplasm—the portion of the cytoplasm outside of the spores—is very vacuolate, except at the periphery of the ascus. The centrosome is conspicuous and stains red. Figure 14 represents a slightly oblique cross-section through an ascus soon after the spores are delimited. The spores are still peripherally located with the centers pointing toward the plasma membrane of the ascus, but not touching it. The membrane of the ascus is intact. The central portion of the epiplasm is vacuolate with delicate thread-like strands of cytoplasm throughout. The nuclei with their beaks are in contact with the spore membrane. The nucleole and the chromatin granules are visible in the wider portion. The center is distinct.

The ascus continues to grow larger. The spores rotate soon after delimitation so that the central body points downward and outward. The beaked nuclei are still in contact with the spore membrane. Figure 15 shows this condition. The ascus is cut longitudinally. The section represented is near the periphery of the ascus. The centers are pointing outward and toward the base of the ascus. The membrane is distinct. The epiplasm is vacuolate throughout the part containing the spores. Clearly defined strands run between the spores and out to the peripheral layer in the ascus. The spores then become much elongated. At the same time the nucleus leaves the spore membrane. The central body is in contact with the nuclear membrane. The nucleus becomes more or less ellipsoidal with the central body toward the lower end and the nucleole at the opposite end. The membrane of the spore is still very delicate. The cytoplasm is less dense than in the earlier stages after delimitation. This condition is shown in figure 16, which represents a small part of an

oblique longitudinal section through the ascus. It was drawn especially to show the nuclei at different distances from the lower end of the spore. The spores vary considerably in this respect. In some the nucleus has just left the lower end; in others the nucleus has almost reached the upper end. This difference is not due to the fact that parts of the spores are cut off. A comparison of the size of the different spores will help to make this clear. The spores have elongated and are cigar-shaped. The nuclei are ellipsoidal in form. The nucleole is on the side, usually nearest the upper end, while the center is at the opposite end, thus showing the same relative position as seen earlier. The chromatin is visible in irregular granules of varying sizes. At this stage the upper ends of the spores extend inward to the central portion of the ascus, often giving the appearance of overlapping from the upper end downward. The spores are separated from one another by a small amount of epiplasm. The epiplasm in the upper and lower end of the ascus still takes up a large portion of the ascus, the spores occupying the central portion between. The plasma membrane is still intact.

As the spores increase in size the ascus grows both in diameter and in length. The spores fill a comparatively large portion of the ascus. As the nuclei reach the upper part of the spores, the spores increase in length. At this stage the nuclei are near the center of the spores where the latter are slightly narrower than at either end (fig. 17). The epiplasm in the upper part of the ascus is shown in this figure. It is still finely granular as in the earlier stages. That in the lower portion is much more vacuolate.

The nucleus moves nearer the upper part of the spore, which increases in diameter. The spore is then spherical with a long tail. The nucleus is found in the spherical portion near the place where it connects with the tail. Figures 18 and 19 respectively show these stages in development. The nuclei are spherical in shape and are oriented in various ways. The nucleole and chromatin are distinct. The center is visible in some of the well-stained preparations. The vacuolate epiplasm surrounding the spores is unchanged. The intimate contact of the epiplasm and the spores is marked in figures 18 and 19.

The upper spherical portion of the spore grows rapidly. The nucleus moves toward the center of the spherical part. Figure 20 shows a section at this stage. The cytoplasm is more vacuolate.

After considerable increase in size there is laid down a thick black wall around the upper portion of the spore which is ovoid in shape. The lower tail portion remains hyaline. It is separated from the larger portion by the thick black wall. The cytoplasm in the upper portion stains darkly, while that in the tail portion stains lightly. Figure 21 shows four spores from an ascus after the spore walls are thickened. The spores appear to be mature. The tail is entirely separated from the rest of the spore by a heavy wall. The nucleus occupies the central portion of the spore. The cytoplasm contains some large vacuoles. The tails are hyaline and extremely inconspicuous in comparison with the heavy black spore walls. The cytoplasm stains very lightly. Often near the distal end of the tail in a hyaline spot are found some dense red staining granules. The epiplasm, though comparatively inconspicuous at this stage, is still present, and the plasma membrane of the ascus is intact.

Discussion

The abundance of well-fixed material of *Philocopra coeruleotecta* has made possible a detailed study of the processes in the development of the ascus and spore formation. It has proven especially favorable because of the variety of stages found in a single pyrenocarp.

The nucleo-cytoplasmic relation shows considerable variation. The very young ascus containing the primary nucleus is not at all striking in this respect. The nucleus and cytoplasm do not exhibit any unusual proportion in respect to each other. Immediately following this stage there is a rapid growth in the size of the ascus. The cytoplasm is slightly more vacuolate and has greatly increased in volume. The primary nucleus shows no signs of division for a considerable period. The cytoplasm actually doubles in volume several times while the primary nucleus remains in a resting condition. That part in the immediate neighborhood of the nucleus, however, shows no marked change. Then with further increase in the size of the ascus the primary nucleus divides. A period of successive divisions of the nuclei follows. The divisions follow one another with great rapidity, filling up the central peripheral portion of the ascus. Whether or not there is any relation between this series of rapid successive divisions and the great volume of cytoplasm is a question of considerable interest. If there is a definite relation between the volume of the nucleus and that of the cytoplasm, the quick successive divisions

of the nucleus may serve to bring about a balance of conditions in this respect. Even at the close of the series of divisions there is a comparatively large proportion of cytoplasm as compared with the volume of the nuclei in the cell, but this is soon remedied by the delimitation of the spores, which brings about a relation between the two which appears more balanced from our general observations on nuclei.

It is apparent from the figures of the nuclei at various stages in the development of the ascus that the nuclei, although entering on an apparently resting stage at the close of each successive division, do not undergo a period of growth sufficient to bring them to the size of the resting mother nucleus in any case. With every increase in number, there is a decrease in the size of the nuclei. The contents appear to be similar, but each part is proportionally smaller in size. The divisions apparently follow one another so rapidly that there is no chance for much increase in size before a new division intervenes. It is interesting that the primary nucleus of the ascus remained in a resting condition while the cytoplasm increased to such an extent. This condition may be in some way related to the formation of kinoplasmic substance. The close of the division period leaves the ascus with abundant kinoplasm, most of that of the astral rays going toward the formation of the spore membranes.

There is no marked differentiation of spore-plasm in this form such as is found in very many of the Pezizas. Although the nuclei are located in a definite part of the ascus, the general cytoplasm of that region is not distinctly different from that of the remainder of the ascus. That part of the cytoplasm which immediately surrounds the nucleus does not show a remarkable increase during the rapid growth of the ascus up to the delimitation of the spores. Its dense finely granular appearance is apparent in the various stages of development. Most of this region is included within the spore after its delimitation.

The intimate relation between the epiplasm and the spores is conspicuous. It is probable that the epiplasm plays a very significant rôle in the feeding of the spores during their rapid growth after delimitation. The adjacent spores are not crowded together. They have a definite relation to each other and are surrounded by epiplasm. Whether or not the epiplasm plays any other part has not been determined. It would be interesting to learn whether it is concerned with the interrelation of the spores. It undoubtedly plays a part in

their final extrusion from the ascus. The long period of existence of such a large amount of epiplasm without nuclei is at least suggestive.

It is certain from the study of *Philocopra coeruleotecta* that the spore wall is laid down by deposition from the outer layer of the cytoplasm within the spore and not by the epiplasm. The spore wall is as thick between the hyaline tail and the main portion of the spore as in the rest of the spore, where it is in contact with the epiplasm. The cytoplasm of the spore has doubtless laid down that portion of the spore wall and probably the remaining part.

The study of the stages in the development of the ascus and in spore formation shows the central body to be present at every stage. The sections were not always stained to bring out a well-defined central body, but in the most favorable preparations the central body stained differently from the chromatin material. It is very small but well defined. In the resting nuclei it appears as a small deep red-staining body in contact with the nuclear membrane. As a rule it is to be found on the side of the nucleus away from the nucleole. The central body is especially well defined in the division figures where the chromatin particles are more remote from it. They are found at the poles of the spindle on the sides of the nuclear cavity. The central bodies are so apparent that there is no doubt as to their existence.

The definiteness of the spindle with the central bodies, even in the late divisions, is too remarkable to leave unmentioned. The minute figures are as clear and distinct as the larger figures. This suggests the possibility of finding more clearly defined figures in some of the other fungi, where they are not sufficiently distinct in the preparations so far figured.

The astral rays can be seen radiating outward from the centers into the cytoplasm in different directions. Some of the preparations are not so favorable as others, but in good clear preparations the rays stand out distinctly. They are very fine, delicate, and numerous. In the fourth nuclear division (fig. 8) a large number of rays are seen in the plane in which the figure is drawn. Figures 6 and 10 show clearly defined asters at the poles of the spindle. Not so many rays are seen in the plane of the figures, but the asters at the poles are unmistakable. There are a number of rays present and the cytoplasm in the immediate vicinity of the central body is fine and dense. The late divisions with a large number of nuclei show conspicuous asters.

At the close of the last division the nuclei are beaked with the

center at the tip of the beak and the astral rays radiating outward in all directions. The divisions take place near the wall and the centers ultimately appear to be in contact with the plasma membrane of the ascus.

The appearance of the distinct beak on the nucleus at the close of so many successive divisions is significant. In the few-spored ascus the beaked nuclei are conspicuous at the close of the third division. The beaks are unmistakable even in the very small nuclei in *Philocopra coeruleotecta*. The central body against the plasma membrane, with the clear-staining nuclear contents, and the relatively long thin beak, are all distinct in appearance.

The beaked nuclei with the centers against the ascus membrane, together with the strongly developed astral rays suggests at once that the delimitation in this many-spored form agrees with that found in the few-spored Ascomycetes. The interastral zones where the rays from adjacent asters meet is further evidence in this direction. Besides, some of the delicate fibers are visible as they turn downward over the nucleus. The long duration of the connection between the center and the spore membrane leaves no question that the spores are delimited by the astral rays as in the few-spored Ascomycetes.

A comparison between the processes here and those in *Geoglossum glabrum* shows them to be remarkably similar in the two cases. The beaked nuclei with their centers in contact with the ascus membrane, the conspicuous asters, the interastral zones, the turning downward of the tips of the newly delimited spores while the centers with the beaked nuclei still remain in contact with the spore membrane, all point toward a similar method of spore delimitation. This method was first described by Harper and is common in the few-spored forms of the Ascomycetes.

Lewis (19) described spore formation in *Pleurago zygospora*, a sixteen-spored Ascomycete. The delimitation occurs at the close of the third division, the same as in the eight-spored forms. A subsequent division produces the sixteen spores. The larger number of nuclei formed in *Philocopra coeruleotecta* before delimitation brought forth the question whether the method of delimitation is different from that described for the other Ascomycetes. Whether or not this method of delimitation is common to the other many-spored Ascomycetes is not yet determined. But the studies of Lewis (19) and Overton (22), as well as the present investigation, suggest that

delimitation takes place in the same manner in all of the Ascomycetes. In some cases division may increase the number of spores. In other instances the nuclei may divide many times before delimitation takes place.

The ascus can in no way be compared with the sporangium in the Phycomycetes. It is probable that further work would reveal still greater uniformity in the process of spore formation in the Ascomycetes. Faull's assumption that the spores are delimited by the method found in the sporangia of the Phycomycetes finds no support in the many-spored Philocopra, where the process has been followed in detail.

There is apparently a direct relation between the nuclear divisions in the ascus and the position of the spores. The first division is either crosswise of the ascus or obliquely so, thus bringing the two resulting daughter nuclei nearer the ascus membrane. The succeeding divisions take place near the periphery of the ascus. The later divisions are nearer the membrane. At the close of the division period the beaked nuclei with the central bodies come in contact with the ascus membrane and the spores are delimited.

The important rôle played by the center and the nucleus in spore formation is apparent from their location near the region of greater activity. At the time of delimitation the nucleus and center are in contact with the ascus membrane. They are in contact with the spore membrane while the spore turns so that they point downward and outward. As the spore elongates they move back from the membrane to the opposite end, which increases in size and becomes the main body of the spore. The constant presence of the nucleus, with the central body, at the point where the main changes occur, suggests that they play more than a passive rôle in spore formation.

Summary

1. The study of a many-spored Ascomycete is important for the determination of the true nature of the ascus. *Philocopra coeruleotecta* Rehm, a new species, is well suited for the study because the spores are comparatively large and the asci are found at various stages in the same pyrenocarp.

2. The cytoplasm in the ascus increases to a considerable extent before nuclear division begins.

3. The first division spindle lies crosswise of the ascus. This

brings the daughter nuclei nearer the ascus membrane. The nuclei in the following stages are near the periphery of the ascus.

4. Successive nuclear divisions follow one another rapidly until one hundred and twenty-eight nuclei are formed. The increase in number of nuclei is accompanied by a decrease in size.

5. At the close of the last division the nuclei are beaked. They are peripherally arranged in the central part of the ascus. The interior of the central part, the proximal portion, and the distal portion of the ascus contain cytoplasm devoid of nuclei.

6. The beaked nuclei, with the central bodies, come in contact with the plasma membrane.

7. So far as observable spore delimitation occurs by the bending back of the astral rays and their subsequent fusion to form the spore membrane.

8. The central body is present at all stages from the young ascus to the mature spore.

9. The delimitation of the spores separates the cytoplasm into the spore-plasm, which is the dense part immediately surrounding the nuclei, and the epiplasm, which fills the remaining part of the ascus.

10. At the time of delimitation the spores are ovoid in shape. The beaked nucleus and central body point toward the ascus membrane. Soon they turn around so that the central body and beaked nucleus point downward and outward. The spores elongate and grow obliquely upward toward the center of the ascus.

11. The central body and the nucleus are always in close relation to the region of greatest activity. After delimitation when the spores elongate, they leave the spore membrane and move toward the opposite end of the spore. The latter grows and becomes the main part of the spore. It is spherical in shape. The lower part remains long and slender and forms the tail of the spore.

12. A thick wall is laid down around the outside of the spherical portion of the spore. This is laid down by the spore-plasm. The epiplasm could not have played any rôle in the process since the wall between the tail portion and the main body is at least as thick as that of the rest of the spore wall. Yet the epiplasm was not in contact with the spore at this point.

13. There is no indication of any phylogenetic relationship between the ascus and the sporangium of the Phycomycetes. The many-

spored ascus does not show a process of spore formation intermediate between that of the sporangium and the ascus. The spores are delimited by the fusion of the astral rays as in the few-spored forms of the Ascomycetes.

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EXPLANATION OF PLATES IX–XI

The accompanying figures were made with the aid of a camera lucida. The magnifications are as follows:

Figure 1; 115 diameters.

Figures 2, 5, 6, 8, 10, 12, 14–16, 18, 19, 21; 1250 diameters.

Figures 3, 4, 7, 9, 11, 13, 17, 20; 1400 diameters.

PLATE IX

FIG. 1. Longitudinal section through pyrenocarp showing three asci and paraphyses.

FIG. 2. Very young ascus with primary nucleus.

FIG. 3. Young ascus, elongated.

FIG. 4. First division figure of primary nucleus of the ascus.

FIG. 5. Ascus cut obliquely. Four-nucleate stage.

FIG. 6. Portion of longitudinal section of ascus showing one of the spindle figures of the third division.

FIG. 7. Eight-nucleate stage. Three nuclei in the section drawn.

PLATE X

FIG. 8. Dividing figure of fourth-nucleate stage. Well-developed asters are present.

FIG. 9. Section of ascus in 16-nucleate stage.

FIG. 10. Dividing figure from many-nucleate ascus.

FIG. 11. Longitudinal section through ascus after last division. Nuclei beaked and peripherally arranged with the centers toward the ascus membrane.

FIG. 12. Cross-section of ascus after the last division. Nuclear beaks directed outward.

FIG. 13. Oblique longitudinal section showing the interastral zones.

FIG. 14. Cross-section of ascus. Spores delimited.

FIG. 15. Longitudinal section of ascus with delimited spores. Nuclear beaks, with the centers, point downward and outward. Epiplasm vacuolate.

PLATE XI

FIG. 16. Spores elongated. Center and nucleus no longer in contact with the spore membrane.

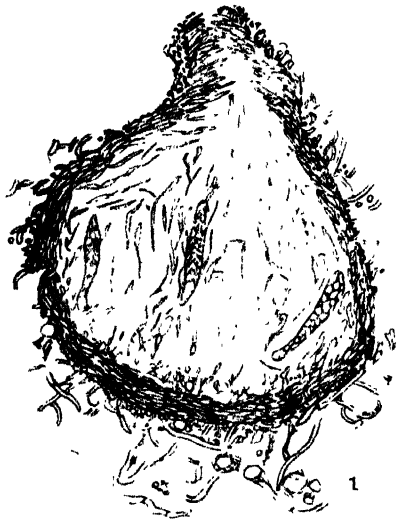
FIG. 17. Nucleus near the center of the spore.

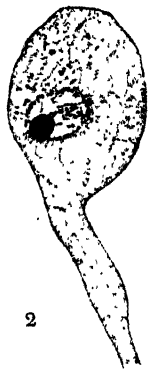
FIG. 18. Spores larger at the upper end. Nuclei in or near the upper portion.

FIG. 19. Slightly older stage. Epiplasm distinct.

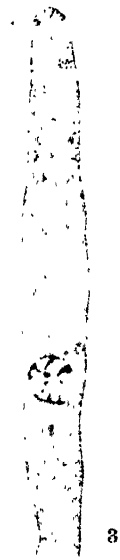
FIG. 20. Spores with large upper part. Nuclei in center of the spherical portions of the spores.

FIG. 21. Spores mature. Spherical portion enclosed by a thick wall. Tails hyaline.















SAX: SPORE FORMATION IN PHILOCOPRA.

















SAX: SPORE FORMATION IN PHILOCOPRA.


16
17
18
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21

SELECTED CYCLES IN GYMNOCONIA PECKIANA

GEORGE F. ATKINSON

At the present time *Gymnoconia peckiana* is one of the most interesting species of the rust fungi. As a result of Tranzschel's cultures it was generally accepted as a short-cycle species with two generations in the complete life history. A disquieting factor was introduced when Kunkel presented the results of his studies on the germination of the aecidiospores, demonstrating a still shorter cycle, since the aecidiospores germinated in the manner normal for teleutospores, *i. e.*, by the production of promycelia and sporidia.

This seeming contradiction in the diverse accounts of the life history of *Gymnoconia peckiana* was interpreted in different ways. Some students suggested that the aecidiospores could germinate in either one of two ways. First, under certain conditions they might germinate in the usual way for aecidiospores, by the production of an infection tube, forming a di-energed mycelium in the host, terminating in the formation of teleutospores, thus presenting a *two-generation cycle*. Second, under other conditions the aecidiospores might assume the functions of teleutospores by germinating with promycelium and sporidia, thus presenting a *one-generation cycle*, of course leaving the spermogones out of consideration.

According to another interpretation, these two different cycles represented two distinct specific, or generic, entities. In these two entities, the aecidial stage of the one-generation cycle and the aecidial stage of the two-generation cycle were morphologically indistinguishable, though specifically distinct. The morphological identity of the aecidial stage of both cycles has been demonstrated by Kunkel in a more recent paper, though I do not understand that he has expressed any opinion as to whether or not the one-generation form is specifically distinct from the two-generation form.

A few years after Tranzschel's cultures, indicating the genetic connection of *Caeoma interstitialis* with *Puccinia peckiana* in Russia, as a matter of personal curiosity I transplanted to the greenhouse some raspberry plants which were free from the Caeoma. On these I sowed

a large quantity of the aecidiospores of *Caeoma nitens* Schw. In the course of six weeks pustules of *Puccinia peckiana* appeared on a few of the leaves. No further attention was given to observations on this rust until June, 1915.

As a result of Kunkel's studies, I was led to test the germination of the aecidiospores in the vicinity of Ithaca, N. Y. In the suburbs of Ithaca, on Cornell Heights along Fall Creek Drive, there has existed for a number of years a rather large patch of the common dewberry (*Rubus villosus* Ait.), which for several years has been very badly affected with this rust. Aecidiospores from the leaves were dusted on the surface of water in several glass vessels, ranging from 10 to 20 cm. in diameter. The spores were so numerous that they formed a dense orange-yellow layer on the surface of the water. In eighteen to twenty-four hours vast numbers of the aecidiospores had germinated with typical promycelium and sporidia. Similar sowings of aecidiospores from the same patch of dewberry plants were made during the month of June, 1915, with the same results.

Some interesting observations were made at that time on the germination of some large aecidiospores. These aecidiospores germinated in two ways. In some cases two distinct promycelia, each bearing four sporidia, issued from each aecidiospore. In other cases a single germ promycelium issued from the aecidiospore, and at a short distance branched into two promycelia each bearing four sporidia. Similar large aecidiospores were found to contain four nuclei, while those of ordinary size contain but two. We are warranted, therefore, in drawing the inference that the four nuclei in the large aecidiospores represent two conjugate pairs of nuclei.

Early in the spring of 1915, I obtained from a nursery in Geneva, N. Y., several young raspberry and blackberry plants free from the disease. These were potted and the pots sunk in the ground in the partial shade of some trees on the south side of my office. Aecidiospores from the dewberry plants were sown on some of these experimental raspberry plants, on which new shoots were just appearing, many of the aecidiospores falling on the soil close against the stems. No evidence of any infection was observed during the season of 1915, either in the form of Caeoma pustules or of teleutospores. In June, 1916, however, one new shoot from one of the raspberry plants bore several leaves with the caeoma richly developed. Apparently the mycelium did not extend to other parts of the system and become

perennial, for there was no evidence of the Caeoma in 1917, on any of these experimental dewberry plants.

One of the important problems still awaiting attack was the question as to whether or not the raspberry Caeoma with the one-generation cycle is biologically the same as the raspberry Caeoma with the two-generation cycle. Tropical and subtropical regions appear to be richer in rusts with the *Endophyllum* type of germination than do the temperate or subarctic regions. *Caeoma nitens* has long been known to have a much farther southern distribution than *Puccinia peckiana* and the recent interesting paper by Arthur clearly shows this. In consideration of these facts, an experiment was made, in June, 1917, on the supposition that temperature might be, to a large extent, the controlling factor, determining the mode of germination of the aecidiospores of the orange rust of the raspberry. This was taken as a working hypothesis, and the following experiment was carried out.

Four of the experimental raspberry plants were sprayed with water. Aecidiospores from the dewberry plants of the same patch mentioned above were dusted on the leaves. This was done June 27, 1917. Excelsior was packed close around the raspberry plants to support several blocks of ice. Each plant with its ice pack was then covered with a bell jar. These experiments were started at 5:30 P.M. The ice was not all melted in twenty-four hours. More ice was added on the afternoon of June 28, and additional aecidiospores were sown. The plants remained covered until the morning of June 30, when the bell jars and excelsior were removed.

The plants were not closely examined again until August 2, 1917. All four of the hosts showed infection. There were several leaves on each plant with yellowish spots and areas, bearing on the under side the minute projecting masses of teleutospores of *Gymnoconia peckiana*. On some of the smaller leaflets the sori were rather thickly scattered over the entire under surface. The teleutospores examined presented in a striking way the peculiar form characteristic of *Puccinia tripustulata* Pk. (24th Rept. N. Y. State Mus. Nat. Hist., 91, pl. 3, fig. 16, 1872).

During the last week in August, 12 to 15 more leaves on the four experimental plants were found to be richly sprinkled with the teleutosori.

The check raspberry plants were close beside those in the chilled

atmosphere under the bell jars. No attempt was made to keep the aecidiospores from falling on them. In fact some of their leaves were well dusted with aecidiospores, but on none of them did teleutosori appear. For several years I have examined the dewberry plants, in the patch where the Caeoma is so abundant, for teleutosori and have never been able to find any. If the normal history of the Caeoma on these dewberry plants was a two-generation cycle, one would expect to find, on the leaves of the shoots which have outdistanced the perennial mycelium, some teleutosori.

The production of teleutosori on those plants surrounded by a chilled moist atmosphere during the period when the aecidiospores were germinating indicates that, because of the lowered temperature, the aecidiospores, or at least many of them, did not germinate by a promycelium, but by an ordinary germ tube, which entered the host leaf forming a diploid mycelium from which the teleutospores arose. Germination of the aecidiospores of *Caeoma nitens*, therefore, appears to be selective, the mode of germination being determined by temperature conditions. When the temperature is comparatively high, they assume the function of teleutospores.

Gymnoconia peckiana, therefore, is an interesting example of a species in which the life cycle is not permanently fixed. In its more southern distribution, where the temperature, at the time of germination of the aecidiospores, is comparatively high, it is generally (perhaps always) a one-generation cycle species. In its more northern distribution it is often, perhaps usually, a two-generation cycle species. In the intermediate region it has sometimes a one-generation cycle, at other times a two-generation cycle, probably depending on the local and seasonal temperatures at the time of germination of the aecidiospores. The general areas of distribution are, in general, well shown by Arthur, though germinations of the aecidiospores have not been tested over the entire area. Central New York lies in the intermediate climatic zone of the distribution of the species. On the ground of the determinating influence of temperature in the selection of a one-generation or two-generation cycle we would expect the existence here in the vicinity of Ithaca, N. Y., of both cycles. Observation over a period of years has shown that in certain years *Puccinia peckiana* is not uncommon, while in other years it apparently is very rare. No attempt has been made here to correlate the abundance or scarcity of the *Puccinia peckiana* in different years with the temperature conditions. But it would seem reasonable to expect

that when comparatively low temperatures prevail during the period of germination of the aecidiospores, *Puccinia peckiana* would be more abundant, other conditions being equal, and conversely there would be a scarcity of the teleuto stage during years when the temperatures in June and early July were comparatively high.

It will be readily seen that the lack of fixity of the generation cycle in *Gymnoconia peckiana* has an important bearing on the principle, recently adopted by some uredinologists, of recognizing the different generation cycles as representing distinct generic concepts. According to the usual practice, which is also in conformity with the International Code, the *Gymnoconia peckiana* would apply not only to the species in its two-generation cycle, but also in its one-generation cycle. For the aecidial stage alone, if one desired to employ a name which would not include the concept of the teleutospore stage, we have the form species name, *Caeoma nitens* Schw. (or *Caeoma interstitialis* Schlecht.). Common practice as well as the International Code permits this use and there is no necessity for a new generic name to apply to the one-generation form of *Gymnoconia peckiana*.

One of the recent practices in uredinology which has many followers in the United States, and which leads to unnecessary confusion, is the new nomenclature applied to the spore conceptacles, or spore pustules, and also to the spores. According to this new principle, the criterion of morphological structures is not their forms nor place in the cycle, but their cytological behavior. It is always very clear, on the basis of the morphological principle, what the aecidia, or aecidiospores, of *Gymnoconia peckiana* are, and the same is true of the teleutosori and teleutospores. On the basis of the new or cytological principle, the morphological aecidium becomes a cytological *aecium* at comparatively low temperatures, but at comparatively high temperatures it becomes a cytological *telium*, or rather a cytological *aecidioid telium*.

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ASPERGILLUS FUMIGATUS, A. NIDULANS, A. TERREUS N. SP. AND THEIR ALLIES*

CHARLES THOM AND MARGARET B. CHURCH

Wehmer in one of his keys based upon single characters has brought together some short-stalked species of Aspergillus[1] without indicating that the number of correlated characters found might justify more than an arbitrary grouping. He calls this section "Schwachwuchsige" or weak growers in spite of the fact that its best-known members, *A. fumigatus* and *A. nidulans*, are cosmopolitan and aggressive forms. If we substitute for this designation the designation short-stalked Aspergilli with calyptriform heads, we will bring together two green series typified by *A. fumigatus* and *A. nidulans*, and an ill-defined group of species whose colors are given as avellaneus, fawn, cinnamon, or reddish brown but never green. In long-continued culture of certain of the green and yellow-green forms within this group, the color changes in the conidial masses have been followed. Greens, blue greens, and yellow greens may run into each other; any of them may develop dark shades in age which mask the original color, but they do not transform into avellaneus or related colors. Similarly the rosy or cinnamon series do not show any trace of green.

Many cultures of members of this general group have been brought together. Some of these forms are readily aligned with species described in the literature. Others diverge more or less widely. Some of these are either entirely undescribed or so inadequately described as to make identification hopeless. All of these forms have stalks short, rarely exceeding 500 μ in length, and heads usually small in diameter with conidial masses in columns (calyptriform), not as separate chains or masses of chains radiating from the vesicle (radiate).

* Published by permission of the Secretary of Agriculture.

[1] Wehmer, C., Die Pilzgattung Aspergillus in morphologischer, physiologischer, und systematischer Beziehung unter besonderer Berücksichtigung der Mitteleuropaeischen Species. Mém. Soc. Phys. Hist. Nat. Genève 33[2]: 1–156. Pls. 1–5. 1901. This paper is commonly referred to as Wehmer, Monograph (Monogr.).

The conidia found are mostly globose and range in diameter from 2.5 to 4.5 μ.

A species in the avellaneus series which has been intensively studied will be discussed first. The name *Aspergillus terreus* is proposed for this species, which has been under observation for about five years. It was first studied from soil cultures made by Prof. W. M. Esten in Connecticut. It was afterward found in soil cultures by Mr. F. M. Scales in Virginia and California, by Mr. S. A. Waksman in New Jersey, and by Mr. F. C. Werkenthin in Texas. It has been isolated from feces by Mr. G. W. Turesson at Seattle, Washington, from decaying avocado in Florida by Prof. H. S. Fawcett, and by the writers from decaying forage in Kansas, from cornmeal ground in Indiana, from musty tobacco, from waters bottled on the American mainland and in Porto Rico, as well as from numerous chance inoculations. It is readily recognized and not uncommon in routine cultures from decaying and soil-contaminated substances.

Some of the cultures obtained reproduce the morphology and reactions of the strain first studied within the degrees of variation found in successive transfers of the same pure culture. With the accumulation of material, however, we find ourselves with a series of related strains rather than a single organism. These vary in colony characters and in details of reaction but present close resemblances in essential characters which render separate descriptions for most of them impossible, as in the case of the forms of *A. niger*.[2] It is entirely possible that investigation, strain by strain, might show equally conspicuous differences in their activities as among the black forms. A technical description has, therefore, been drawn in broad enough terms to include the more closely related of these forms. Whether some of them may ultimately be separated as varieties, upon physiological grounds, is not determined.

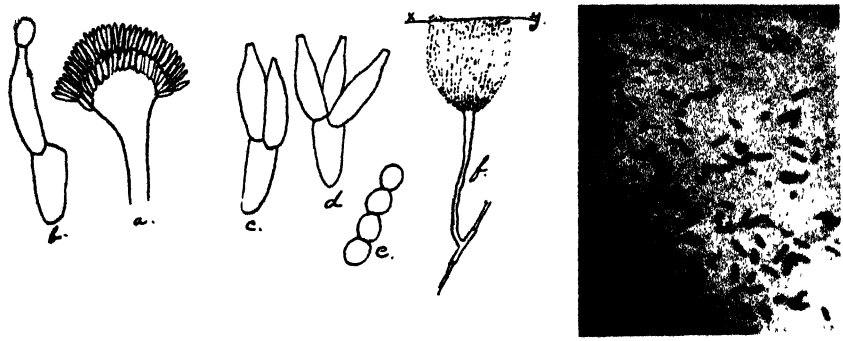
A. terreus Thom[3]

Colonies upon Czapek's solution agar from tints of pinkish cinnamon through cinnamon (at times near avellaneus of Saccardo's

[2] Thom, C., and Currie, J. N. *Aspergillus niger* group. Journ. Agr. Res. 7: 1–15. 1916.

[3] Published without description marked Thom MS. by Göte Turesson in Svensk Botanisk Tidskrift 10: 5 et seq. 1916, in his discussion of "The presence and significance of moulds in the alimentary canal of man and higher animals."

Chromotaxia)[4] to deeper brown shades in age (see Ridgway, Pl. XXIX, 15″. Klincksieck and Valette, Nos. 103D, 112, 113, 108);[5] spreading, velvety or in some strains developing definite floccosity or anastomosing ropes of aerial hyphae; reverse and agar from pale or bright yellow to fairly deep browns. Odor, none in some strains, at least transiently present in others, or developing with the addition of high percentages of cane sugar. Conidiophores 5–8 μ in diameter, 50–150 μ or even 250 μ in length, more or less flexuous, with walls smooth, up to 1 μ thick, septate or unseptate, with apex enlarged to



1

FIG. 1. *A. terreus*. *a*, semidiagrammatic section of vesicle and sterigmata; *b*, *c*, *d*, primary and secondary sterigmata, × 1,500; *e*, conidia, × 1,500; *f*, diagram of stalk and base of calyptrate conidial mass.

FIG. 2. *A. terreus*. Photograph of colony on Petri dish of Czapek's medium.

form a vesicle commonly 12–18 μ, occasionally up to 25 μ in diameter, bearing sterigmata usually in two series upon its dome-like upper surface; primary sterigmata 2–2.5 μ by 7–9 μ, secondary 2–2.5 μ by 5–7 μ closely packed; heads becoming solid columnar masses up to 500 μ long by 50 μ in diameter; conidia slightly elliptical to globose, 2.2–2.5 μ or even to 3 μ in diameter, smooth, in long, parallel, adherent chains. Perithecia not found. Grows at 37° C. Liquefies gelatin.

Habitat.—Common in soil and in decaying vegetable matter, throughout the United States.

Turesson[6] reports the spores of this species as viable after passing

[4] Saccardo, P. A. Chromotaxia seu Nomenclator Colorum, Patavii. 1891.

[5] Ridgway, Robert. Color standards and color nomenclature. Washington, D. C. 1912.

[6] Turesson, Göte. Bot. Tidskr. 10: 1–27. 1916.

through the human digestive tract. This led him to feed cultures of this species to a rabbit, which afterwards died. *A. terreus* was recovered in culture from numerous portions of the intestine (Turesson, loc. cit., p. 20). Further studies upon the possible relation of this form to toxin production are necessary.

Some experiments were made to find whether this form is an active inhabitant of the soil or merely present in spore form. Two forms of soil, a light sandy loam from Texas and a clay type from Indiana, were obtained from the soil fertility laboratory; a third sample was obtained from the greenhouses of the Arlington Farm. Finely divided soil to a depth of about 5 cm. was tamped into test tubes and sterilized. Part of the series was given fractional sterilization in steam, the remainder 30 minutes in the autoclav at 15 pounds pressure. Three cc. of water were added to the light soil and 2.7 cc. to the heavy soil. The water was added before heating, in the case of the fractional sterilization, but after heating, in the case of autoclaving the tubes. These differences of manipulation seemed to have no effect upon the growth of the organisms tried.

The test tubes of soil were inoculated by sprinkling spores on the surface of the soil in the test tubes. For comparison of growth, three strains of *A. terreus*, one of *A. nidulans*, six of *A. flavus*, one of *A. clavatus*, one of *A. oryzae*, three of the Citromyces section of Penicillium, *P. pinophilum* and *P. luteum*, were used. Each is representative of a group of related forms repeatedly found in soil. After a period of approximately two months, the typical conidial masses of *A. terreus* could be seen not only upon the surface but in the open spaces in the sandy soil to a depth of 3 cm. The organism was recovered in pure culture from the deepest areas, where growth was not visible, by breaking the tips of the test tubes and transferring some particles of soil to culture media. Although cultures showed the mold to be present, traces of mycelium were often very difficult to find by microscopic examination. Mycelia under such conditions are much less evident. Short zigzag hyphae are found in intimate contact with soil particles rather than richly branching mycelia ramifying through wide areas. Spores are produced from short stubby branches extending into small open spaces. Hyphae and heads under these conditions are not recognizable by data based upon pure culture in artificial media. Nevertheless, it is clear from the table that *A. terreus* and many others of these species planted upon its surface are

capable of growing into soil to considerable depths and even capable of producing spores under conditions in which many fungi fail to fruit at all.

The tabulated data (Table I) from these comparative cultures show that these organisms, which were selected because they are constantly obtained in studies from the soil, are capable of actively growing within the soil.

TABLE I

Comparative Cultures of Organisms in Different Kinds of Soils

Name	Race	Clay Soil, Depth in Cm.		Sandy Soil, Depth in Cm.		Greenhouse Loam, Depth in Cm.	
		Spores Visible	Recovered, in Culture	Spores Visible	Recovered, in Culture	Spores Visible	Recovered, in Culture
A. castaneus	3565	*	*	Surface	5 cm.	Surface	
A. clavatus	4083	*	*	*	*		5 cm.
A. flavus	128		5 cm.	2 cm.	5 cm.		3.5 cm.
" "	1763	*	*	*	*	Surface interstices	o at 5 cm.
" "	2750		1.5 cm.	1.5 cm.	5 cm.		4 cm.
" "	3557.6	*	*	*	*	Surface	5 cm.
" "	3557.9	*	*	*	*		4 cm.
" "	4006.2	*	*	*	*		4.8 cm.
A. fumigatus	118	Surface	5 cm.	5 cm.	5 cm.	*	*
" "	2496	Surface		*	*	*	*
A. nidulans	4010.4	*	*	5 cm.	5 cm.	*	*
A. oryzae	113		Bottom of tube 5 cm.	Surface	5 cm.		4 cm.
A. terreus	144	*	*	3 cm.	3 cm.	*	*
" "	Ra42		5 cm.	Surface	5 cm.		4 cm.
" "	3533	Surface	5 cm.		5 cm.		o at 5 cm.
Penicillium (Citromyces)	2467	*	*	Interstices	5 cm.	*	*
Penicillium (Citromyces)	4019.2	*	*	Interstices	5 cm.	*	*
Penicillium (Citromyces)	4083		Bottom of tube 5 cm.	5 cm.			o not at 4.3 cm.
P. luteum	11	*	*	2.8 cm.	5 cm.	*	*
P. pinophilum	1		Bottom of tube 5 cm.	5 cm.	5 cm.	Surface	5 cm.

* No information.

The literature was searched to find a name and description applicable. In form and habit of colony *A. terreus* resembles *A. fumigatus*

Fres. and *A. nidulans* Eid., both of which are abundant in soil cultures in America. Both of these species are green; *A. terreus* is never green. All three forms have small spores (2.5 μ to 4 μ), short stalks and dense columnar or calyptriform masses of conidia. They thus have the form given for *A. calyptratus* by Oudemans.[7] *A. fumigatus* has one set of sterigmata in all heads. *A. terreus* shows one set of sterigmata occasionally in young heads but two sets in well-developed heads. *A. rehmii* has double sterigmata but is described as yellow. In *A. calyptratus* the conidial column although described as black is so colored in Oudemans's figures as to suggest *A. terreus*. More recently Werkenthin[8] identified one strain of *A. terreus* as obtained from soil in Texas with *Sterigmatocystis veneta* of Massalongo.[9] This form is described as having "fasciculate fertile hyphae" and to be in color pale or dirty yellowish ("pallide vel sordide luteolis"). The Texas strain has superficial, interlacing, trailing ropes of hyphae from which short conidiophores arise. The same condition is produced in our original strain of *A. terreus* when grown upon peptone-beef-juice agar with cane sugar. The description of Massalongo does not appear to justify this identification.

A considerable number of species have been described in color as avellaneus, cervinus, cinnamomeus, roseus, or by technical names falling within this related series of colors. These color-terms have been used so vaguely as frequently to mean only that the color so designated comes into the group, not that it has a definite tint or shade. Cultural study, moreover, shows that the same strain when grown under a series of differing cultural conditions may be successively described by a whole series of these names. These variations have been studied in detail for certain series of forms with reference to the Code de Couleurs by Klincksieck and Valette[10] and the recent work of Ridgway.[11] While exact duplication of culture-color in the charts is rare, the variations within closely related series tend to fall in the columns of Ridgway's plates; that is, in tints and shades of

[7] Oudemans, C. A. J. A., and Koning, C. J. Prodrome d'une flore mycologique obtenue par la culture sur gelatine preparée de la terre humeuse du Spanderswoud près de Bussum. Extr. Arch. Neerl. Sci. 267–298, pls. 1–41. 1902.

[8] Werkenthin, F. C. Fungous flora of Texas soils. Phytopathology 6: 241–253. 1916. Ref. to pages 247–248–249.

[9] Massalongo, C. Novita della flora micologica Veronese. Bull. Soc. Bot. Ital. 259. 1900.

[10] Klincksieck, P., et Valette, Th. Code de Couleurs. Paris. 1908.

[11] Ridgway, loc. cit.

single mixtures of primary colors. The young culture may first show color as one of the paler tints of a series, then become successively deeper, and not infrequently in age change to some one of the darker shades of the same series. A change in the primary mixture frequently develops in the same colony with age. Such changes with rare exceptions bring combinations closely related to the original, such as are found in adjacent columns or upon the same page in Ridgway.

An organism showing one of these colors must, therefore, be critically compared in all its characters with those described as showing any of the related colors. In making this comparison these descriptions have been brought together and considered. Before describing *A. terreus* as new the original descriptions of this series were examined in every case and frequently all references in the literature were followed. The original citations have been included in the synopsis of the group presented and where possible an opinion is given upon the proper placing of the form.

In habit and colony appearance *A. terreus*, *A. fumigatus* and *A. nidulans* resemble each other more closely than they resemble such forms as *A. niger*, *A. ochraceus* or *A. flavus*. They may, therefore, be taken as typical forms in three related series. A brief review of the history of the two series of forms typified by *A. fumigatus* of Fresenius[12] and *A. nidulans* of Eidam[13] will be followed by a synoptical presentation of the whole group as far as the material could be interpreted.

Aspergillus fumigatus Series

A. fumigatus was described by Fresenius in 1850. References to molds in the human ear go some years farther back, but no previous author gives an adequate description of the form. The figures of Fresenius fix a type of conidiophore and fruiting head which is readily found by examination of cultures today. However, since organisms with this morphology are found everywhere and upon a wide variety of substrata, the student of comparative cultures soon finds strains with this conidial morphology but cultural characters diverging fairly widely and apparently fairly stable. It is not surprising to find

[12] Fresenius, J. B. G. W. Beitrage zur Mykologie. P. 81, pl. 10, figs. 1–11. Frankfurt. 1850.

[13] Eidam, E. Zur Kenntniss der Entwickelung der Ascomyceten. Cohn's Beitr. Biol. Pflanzen 3: 377. Pl. 21, 22. 1879.

some of them described as separate species. This type of organism is reported from the tongue, the ear, the cornea of the eye, and the human lung. As a cause of aspergillosis in birds, it is found in the lungs of various species. Cultures have been extensively tested and found pathogenic in varying degrees to fowls, rabbits, guinea pigs, and dogs. We have received it from soil bacteriologists working in widely separate regions, and recovered it many times from forage and musty or moldy grains. This type of mold has proved to be a regular inhabitant of soil at least in America, hence may be expected in cultures from any substance contaminated with dirt. All of these strains grow at the temperature of warm-blooded animals, this being a prerequisite to pathogenesis. The pathological literature with reference to *A. fumigatus* is extensive. The literature of the group was critically examined in 1905 by Costantin and Lucet[14] who recognized the close relationship of the whole series of forms. They retained as species *A. malignus* Lindt, *A. bronchialis* Blumentritt, *A. lignieresi* Cost. & Lucet, *A. fumigatus* Fres., *A. virido-griseus* Cost. & Lucet, and *A. penicilloides* Speg.

They regarded the other forms already described as synonyms or unrecognizable. Comparison of long series of cultures from different sources confirms belief in the ability of races or strains to maintain specific cultural characters in *A. fumigatus* as has been already described for *A. niger* (Thom and Currie, loc. cit.). Some of these forms can probably be described in morphological and physiological terms which will identify them. It is probable, however, that the number of races showing at least slight differences is very much greater than these investigators believed and that by sufficient search connecting forms would be found which would make up a fairly complete series. The determinations of pathogenicity already reported (Cost. and Lucet, loc. cit.) show that the strains so far studied vary markedly in this respect. Physiological differences of marked degree are not necessarily correlated with morphological characters. This has been demonstrated for *A. niger* by Thom and Currie, and is clearly shown in the studies of relative pathogenicity of *A. fumigatus* as reported by Costantin and Lucet.

Perithecia have been reported for *A. fumigatus* by Behrens[15] and

[14] Costantin, J., et Lucet. Recherches sur quelques Aspergillus pathogènes. Ann. Sci. Nat. Bot. IX. 2: 119–180, pl. 5. 1905.

[15] Behrens, J. Centralbl. Bakt. 11: 335. 1892.

by Grijns.[16] The validity of both of these determinations is disputed by Vuillemin[17] who probably correctly regards the perithecia found by Behrens as belonging to the *A. glaucus* series and makes those described by Grijns the basis of *A. pseudo-nidulans* Vuillemin. On the other hand, the descriptions of perithecia for *A. fumigatoides* by Bainier and Sartory[18] and for *A. fischeri* by Wehmer[19] are scarcely questionable. Both of these forms reproduce the conidial form of *A. fumigatus* within the limits of variation reported for this form

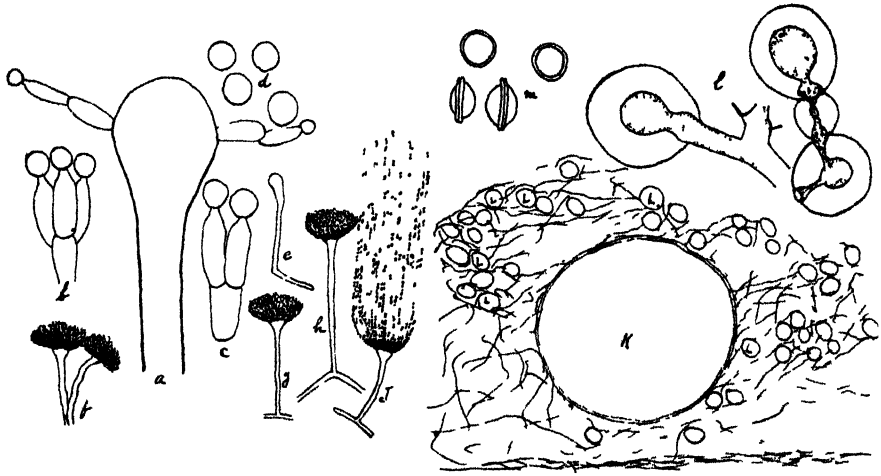


FIG 3 *A nidulans* (American soil strain no 131) *a*, diagrammatic section of vesicle with two sterigmata, *b*, *c*, primary and secondary sterigmata, × 1,500; *d*, a group of conidia, × 1,500, *e*, *f*, *g*, *h*, *j*, diagrams of stalks and heads; *k*, perithecium surrounded by sterile hyphae and Hulle-cells (*L*), *l*, Hülle-cell enlarged showing the thick walls and the granular cell contents, *m*, a group of ascospores.

as it is found causing aspergillosis of birds. We have recently studied a form obtained from two separate sources in which the conidial areas were much reduced when cultivated at 20°–25° C. but developed characteristic areas of the *A. fumigatus* type at 37° C. Perithecia in both strains begin to appear abundantly within the first few days on Czapek's solution agar but not at all in plain agar (beef-extract-

[16] Grijns, G. Die Ascusform des Aspergillus fumigatus. Centralbl. Bakt. II. 11: 330–332. 1903

[17] Vuillemin, P Archiv de Parasitologie 8 540. 1904.

[18] Bainier, G., et Sartory, A. Bull. Soc. Myc. France 25: 112, pl. 5. 1909.

[19] Wehmer, C. Centralbl Bakt II. 18 390–393, figs. 5 1907.

peptone agar of the bacteriologists). They originate in coiled hyphae similar to the process described for *A. fumigatoides* or by DeBary[20] for *A. repens*. The measurements and markings are a composite of those of *A. fischeri*, *A. fumigatoides*, and *A. malignus* Lindt. Clearly these forms are closely related and just as certainly may be regarded as members of the *A. fumigatus* group. How many of the entire series will stand critical examination is still in doubt. With this group as in previous papers (Thom, Thom and Currie),[21] it seems best to retain in the literature of the series the specific names found applied to such well-described forms as may represent in typical manner particular lines of variation. In general, however, it must be recognized that all the forms thus far reported grow at 37° C. or higher, that all of them as far as tests have been reported in the literature have proved pathogenic to some of the usual experimental animals, that the conidial apparatus in all of them corresponds closely to the description by Fresenius. Even in ascospore production the common characters found overshadow the differences which are limited to contrasts in size and in details of spore markings. (See synopsis of the group, p. 97.)

Characterization from Cultures (see also Table 2).— Colonies on Czapek's solution agar in some strains strictly velvety, in others with varying amounts of tufted aerial mycelium up to felted floccose forms, green to dark green, becoming almost black in age, spreading. Reverse and substratum, in some strains uncolored, in others showing varying amounts of yellow, this occasionally becoming reddish in age. Conidiophores short, usually densely crowded, up to 300 μ (occasional strains to 500 μ) long by 2–8 μ in diameter, frequently more or less green colored, especially in the upper part, arising directly from submerged hyphae or as branches from aerial hyphae, septate or unseptate, gradually enlarged upward, with apical flask-shaped vesicles up to 20–30 μ in diameter, fertile only on the upper half, bearing sterigmata in one series, usually about 6–8 μ (varying from 5–10 μ) by 2–3 μ, crowded, closely packed with axis parallel to axis of the stalk; chains of conidia form solid columns up to 400 μ by 50 μ, but usually much shorter; conidia dark green in mass, globose, 2–3.5 μ

[20] DeBary, A. Eurotium, Erysiphe, Cincinnobolus nebst Bemerkungen über die Geschlechtsorgane der Ascomyceten. Beitr. Morph. Phys. Pilze 3: 1. 1870.

[21] Thom, C. The Penicillium luteum-purpurogenum group. Mycologia 7: 134–142. 1915; Thom, C., and Currie, J. N., loc. cit.

mostly 2.5–3 μ in diameter. Perithecia not found in most of the strains investigated, abundantly produced in certain strains [data from No. 4188.21] up to 300 μ in diameter, not colored, or very pale salmon, with walls scarcely colored, consisting of a single layer of cells, crushing easily, covered by a loose network of uncolored sterile hyphae; asci abundant, filling the perithecium within a few days, from 8 μ by 10 μ to 10 μ by 12 μ, subglobose, breaking down quickly to leave the perithecium full of ripe ascospores; ascospores biconvex, 7 μ by 4 μ, consisting of a central body 5 μ by 4 μ, with two frilled equatorial bands about 1 μ in width and 3 to 4 similar but narrower and anastomosing bands on each convex surface, separating into 2 valves in germination.

All strains grew over a wide range of temperature, better at 37° C. or higher than at lower temperatures. A few strains produce green conidial areas only at high temperature.

Habit.—Variable and widely distributed in soil and soil-contaminated substances, on forage, and on grain, as a cause of aspergillosis in birds.

TABLE 2

Comparative Measurements of Aspergillus fumigatus and Allies

Name	Habit	Stalk	Vesicle	Sterigmata	Conidia
A. aviarius	*	7.5†	20–30	*	2–2.5
A. bronchialis	*	280–300 x 9	12–19	*	3–4.2
A. fischeri	*	300 x 6–7	20	5.7 x 2.5	2.4–3.5
A. flavo-viridescens					
A. fumigatoides	*	150–310 x 5–2	30–35	8–14 long	2–3 x 2
A. fumigatus	*	150–340	16–30	*	2.5
A. f. var. tumescens					
A. gracilis	Floccose	250 x 2.8	24	5–6 long	3 ave.
A. gracilis var. *exiguus*	*	–3 diam.	20–35	3–6 long	*
A. keratitis	No adequate description.				
A. lignieresi	Floccose	180–230 x 6.8	–24	–6 long	2.4–3
A. malignus	*	–1000 x 3	22–24	10 x 4–4.5	3–4
A. olivaceus	Data entirely lacking.				
A. penicilloides	*	–100 x 4.5	10–12	3–4 x 2.5	–3
New Orleans Culture		–200 x 3–7	10–12	–8.5 x 2.5	–3
A. pusillus	Floccose	50–74 x 3–4	10–12	3 x 1?	–1?
A. quininae	*	6–7 diam.	*	*	–3.5
A. ramosus	Hallier's figures are the only clue to identity.				
A. viridogriseus	Floccose	400–600 x 6–15	25–36	–5 long	–2.8

* No information.

† All measurements given are in micromillimeters.

ASPERGILLUS NIDULANS SERIES

Eidam (loc. cit.) in 1880 described *A. nidulans* as obtained from the nest of some type of wasp or bee in the botanical garden at Breslau. Conidial forms corresponding to Eidam's figures and description have been found in many situations since that time. Members of this series have been shown to be pathogenic, by animal inoculation. As far as tested, they all grow at 37° C. or higher. Saito[22] has reported them from the air in Japan. *A. nidulans* var. *nicollei* has been described as a cause of disease in man. Forms with this morphology have been repeatedly isolated from soil in various parts of America and by us from soil-polluted substances. This conidial type appears to be cosmopolitan and in America, at least, a characteristic inhabitant of the soil in which experiments show its ability to multiply (see table).

The ascospore of *A. nidulans* repeats the general structure originally described by DeBary[23] for *A. repens*. It is more or less lens-shaped. When such an ascospore germinates the purple cell wall separates into two valves like those of a shellfish. These sometimes remain in contact at one edge but commonly remain attached to opposite sides of the germinating cell as figured by Eidam. Eidam reported no markings upon these ascospores. Grijns found a single equatorial band where the two valves meet. Vuillemin reports this band as double. One of our cultures shows a slight equatorial furrow with traces of a ridge in each side—approximately the form of the ascospore in *A. repens*. In all other cultures observed, there are two definite bands of varying width between which is the line at which the valves separate. These bands and the surface markings on the valves when present have the appearance presented by the wrinkled, folded, or at times closely fitting primary wall of the conidium as described for Penicillium by Thom[24] and for *A. niger* by Thom and Currie.[25]

Aspergillus fumigatus has strictly a single series of sterigmata or conidia-bearing cells upon the vesicle. *A. nidulans*, on the contrary, has both primary and secondary sterigmata in every head, hence has

[22] Saito, K. Untersuchungen über die atmosphärischen Pilzkeime. Journ. Coll. Sci. Imp. Univ. Tokyo 18: 1–58. 1904.

[23] Loc. cit.

[24] Thom, C. Conidium formation in Penicillium. Mycologia 6: 211–215. 1914.

[25] Thom, C., and Currie, J. N. Loc. cit., p. 7

been called a Sterigmatocystis. That usage is disregarded in this paper. Comparative study of these two groups of races, *A. fumigatus* and *A. nidulans*, brings cumulative evidence of close relationship. *Aspergillus rehmii* Zukal and *Sterigmatocystis sydowi* Bainier and Sartory have both been cited as *A. nidulans* but this does not seem to be justified by examination of all the data given. *Aspergillus flavo-viridescens* Hanzawa appears to be more closely related to *A. versicolor* than to *A. nidulans*. *S. glauca*, *S. minor*, and *S. prasina* of Bainier and *S. olivacea* Van Tieghem might have been varieties of *A. nidulans*. The descriptions are inadequate for identification.

Group Characterization of A. nidulans from Cultures.—Colonies on Czapek's solution agar, white to yellowish green, finally fairly deep green, velvety to more or less floccose in purely conidial areas, definitely floccose when perithecia are forming, reverse and agar usually more or less reddish to dark red or reddish brown, conidiophore more or less flexuous with the walls colored in shades of cinnamon brown, septate or unseptate, usually 50–100 μ but up to 200 μ long by about 3–5 μ in diameter, increasing gradually to a dome-like vesicle 7–15 μ in diameter, bearing sterigmata in two series, parallel with axis of the stalk; primary sterigmata varying, 5 by 3 μ to 7–8 μ by 2–3 μ; secondary sterigmata 7–10 μ by 2–2.5 μ; conidia globose up to 3 μ or 3.5 μ, occasionally to 4 μ in diameter, smooth or rough, greenish, in parallel chains adherent into a solid column 30–50 μ in diameter and up to 100–200 μ in length.

Perithecia becoming globose, up to 200–300 μ in diameter surrounded by floccose white to gray mycelium, the branches producing, either terminally or in a terminal series, yellowish to cinnamon globose cells up to 25 μ in diameter with walls 4–5 μ in thickness (the Hülle); perithecial walls thin, brittle, consisting of one or two layers of polygonal cells from pink to deep red, almost black, turning blue with the addition of alkali and red again with acid. Asci pink to purple, numerous, filling the perithecia, 8-spored; asci and ascospores varying in size with the race or species. The following variations may be described:

1. *A. nidulans* Eidam. Asci 10.5–11 μ, ripening slowly over a period of many weeks; ascospores slightly oval, about 5 by 4 μ, smooth with deep purple walls, separating into two valves in germination. A culture with this type of ascospores has recently been found by us among the soil forms isolated by Waksman in New Jersey.

2. *A. pseudo-nidulans* Vuillemin.[26]—Asci 9 by 14 μ; ascospores 4 by 4.5 μ, lenticular, with double equatorial bands, these being extensions near the edges of the valves of the ascospore. Vuillemin regards this as the form which Grijns reports as an ascosporic *A. fumigatus*. The double character of the equatorial band is so distinct that it is difficult to believe that Grijns failed to see it if present. The possibility of finding Grijns's organism with a single equatorial band therefore remains open.

We have three variations of this ascosporic series distinguished as follows:

3. No. 110, received from Dr. Westerdijk at Amsterdam. Asci ripening slowly a few at a time over a period of several weeks, 10–13 μ in diameter; ascospores lens-shaped, about 4–5 μ in diameter and 3–3.5 μ in thickness with a plaited, folded, or wrinkled equatorial band as a free extension of the margin of each valve to a width of 1.5–1.8 μ (the double equatorial band of authors).

4. No. 4110, from flax straw and the same, No. 131, from soil, shows perithecia full of ripe ascospores within a few days; the asci mostly break down quickly, leaving the perithecium full of free ascospores; ascospores measuring as in No. 110, except that the equatorial bands are 1 μ or less in width.

5. No. 4138T11 differs from No. 4110 in the appearance of ridges and folds on the valves of the ascospore. It was obtained by Waksman from New Jersey soil.

SYNOPSIS OF WHOLE GROUP

A. Green series.
 B. Sterigmata simple. *A. fumigatus* series. Characterization page 90.
 C. Relationship to *A. fumigatus* distinct.
 D. Ascospores known.
 E. Ascospores with double band.
 1. Asci 14–18 μ; ascospores 6–8 μ in longest diameter — *A. malignus* Lindt
 2. Asci 10–12 μ; ascospores –7 μ in longest diameter. B. C. No. 4188.21.
 3. Asci 20–26 μ x 12–18 μ; ascospores 3–3.5 μ..... — *A. fumigatoides* B. & S.
 EE. Ascospores with single band.

[26] Cf. *A. fumigatus* Grijns, Centralbl. Bakt. II. 11: 330. 1903

F. Asci and ascospores colorless.
4. Asci 10–12 μ; ascospores 5.6 μ x 4.2 μ . . . *A. fischeri* Wehmer
FF. Asci and ascospores red.
5. Asci 9 x 14 μ; ascospores 4 μ x 4.5 μ. Grijns' strain. (See *A. pseudo-nidulans.*)
DD. Ascospores not found.
G. A. fumigatus Fres. (See type description.)
6. Probable synonyms. *A. quininae* Heim, *A. keratitis* Ball, *A. bronchialis* Blum., *A. aviarius* Peck, *A. ramosus* Hallier, *A. nigrescens* Robin, *A. microsporus* Böke, *A. olivaceus* Preuss, *A. glaucoides* Spring.
GG. Strains separated from *A. fumigatus* by cultural details. *A. lignieresi* Cost. & Lucet, *A. virido-griseus* Cost. & Lucet, *A. fumigatus* var. *tumescens* Blum.
CC. Relationship indistinct or doubtful.
H. Diminutive forms. (*a* and *b* probably not related.)
a. Conidia 3 μ *A. penicilloides* Speg.
b. Conidia 1 μ *A. pusillus* Massee
HH. Vigorous active green colonies . . *A. gracilis* Bainier, *A. gracilis* var. *exiguus* B. & S.
HHH. Descriptions inadequate. *A. Nölting* Hallier, *A. Hageni* Hallier, *A. heterocephalus* Spring, *A. ageni.*
BB. Sterigmata in 2 series.
I. Perithecia known. (*A. nidulans* series.)
K. Asci developed slowly (several weeks).
1. Asci 10.5–11 μ diam. Ascospores without band or frill . *A. nidulans* Eidam
1*a.* Soil culture 4163c28-reproduced Eidam's description.
1b. Var. pathogenic to man . . *A. nidulans* var. *Nicollei* Pinoy

	2. Asci 10–13 μ diam. Ascospores with 2 bands 1.5–1.8 μ in width. (Culture.) Amsterdam strain.	
KK.	Asci developed quickly (a few days).	
	3. Ascospores with a single band	*A. fumigatus* (?) Grijns
	4. Ascospores with 2 bands . . .	*A. pseudo-nidulans* Vuillemin
	5. Ascospores with 2 bands 1 μ or less in width. (Culture.) American soil strain. Probably the same as no. 4.	
	6. Ascospores with 2 bands, also ridges and folds on valves. (Culture.) New Jersey strain.	
II.	Perithecia unknown. Relationships not determinable from descriptions given. *Sterigmatocystis glauca, S. minor, S. prasina.*	
AA.	Colonies never green—some mixture of yellow-orange and neutral gray, avellaneous, clay, cinnamon, etc.	
M.	Forms with 1 series of sterigmata.	
	Heads (cervinus) fawn color (culture)	*A. cervinus* Massee
	Heads rosy.	*A. roseus* Link
	Heads in a dark brown to black column (culture by Oudemans). . . .	*A. calyptratus* Oud.
MM.	Forms with 2 series of sterigmata.	
	Flesh-color	*S. carnea* van Tieg.
	Cinnamon to avellaneous (culture)	*A. terreus* n. sp.
	Coremiform, tufted. . . .	*S. veneta* Massalongo

List of Published Species

The citations of the original descriptions of the species mentioned in this paper are given in alphabetical order by species names. The initial A. or S. indicates that the describer regarded the species as Aspergillus or Sterigmatocystis respectively. Except where indicated the original description has been examined. This list includes some forms not closely related to the species considered in the paper but which have been referred to by authors as belonging with these forms.

A. africanus Durieu & Montagne, Fl. Alg. p. 342. 1849. Reference is made to this description because the form is described as reddish brown; the spores were described, however, as 20 μ in diameter.

A. ageni. This name is cited by Lindt, Arch. Exp. Path. Pharm. **25**: 265. 1889, as taken from Saccardo's Sylloge. Search for this reference leads to the conclusion that in this citation *A. Hageni* was made to read *A. ageni.*

A. aviarius Peck, N. Y. State Museum Rept. **44**: 25. pl. 4. figs. 9–12. 1891. The description of this form leads to the belief that the organism was some strain of *A. fumigatus.*

A. bronchialis Blumentritt, Ber. Deutsch. Bot. Ges. **19**: 442–446. pl. 22. figs. 1–6. 1901; also ibid. **23**: 419–427. pl. 19. figs. 1–3–6–7–8–19–23. 1905. This colony is described as floccose in contrast to the commoner forms of *A. fumigatus* which are velvety or produce very little aerial mycelium. Close relationship to *A. fumigatus* is evident.

A. calyptratus Oudemans, Arch. Neerl. II. **7**: 283. pl. 13. 1902. Conidial chains forming a black column are reported by Oudemans but the mass is figured as brown, thus possibly *A. terreus*, or a species of Haplographium.

S. carnea van Tieghem, Sur le développment de quelques Ascomycetes, Bull. Soc. Bot. France **24**: 103. 1877. Saccardo, Sylloge **4**: 74, and Wehmer, Monogr.: 127. The conidia are given as flesh-color without other data.

A. cervinus Massee, Kew Bull. Misc. Inf. **4**: 158. 1914. A fawn-colored species from African soil with morphology close to *A. fumigatus.* A culture with closely similar characters was contributed by Dr. J. R. Johnston from Porto Rico soil (3522.36).

A. fischeri Wehmer, Centralbl. Bakt. II. **18**: 390–2. fig. 5. 1907. The conidial morphology reported is not different from *A. fumigatus.* Perithecia are described, see p. 93.

A. flavescens Wreden, Compt. Rend. Acad. Sci. Paris **65**: 368–371. 1867. Also, St. Petersb. Med. Zeitschr. **13**: 133–184. 1867. This species has been regarded as related to *A. flavus* but the conidia described are 2–3 μ in diameter and the upper parts of the stalks are described as yellowed. This establishes a strong probability that it was some strain of *A. nidulans.*

A. flavo-viridescens Hanzawa, Journ. Coll. Agr. Tohoku Imp. Univ. Sapporo **4**: 232–3. pl. 21. figs. 1–4. 1911. The description by Hanzawa suggests a closer relationship to *A. versicolor* than to *A. nidulans* as judged by our cultures of both groups.

A. fumigatoides Bainier & Sartory, Bull. Soc. Myc. France **25**: 112. pl. 5. 1909. The conidial apparatus described is hardly distinguish-

able from *A. fumigatus*. Perithecia were found with ascospores differing in detail from *A. fischeri*, *A. malignus* and the form we have described.

A. fumigatus Fresenius, Beiträge zur Mykologie, pp. 81. pl. 10. figs. 1–11. Frankfurt. 1850–53. See Wehmer. Mem. Soc. Phys. Hist. Nat. Genève **33**: 70. 1901. Perithecial form described by Behrens, J. Centralbl. Bakt. **11**: 335. 1892, and by Grijns, Centralbl. Bakt. II. **11**: 330. 1903. Vuillemin, Arch. Parasit. **8**: 540. 1904, decides that asci have never been found in *A. fumigatus* and that Grijns was dealing with *A. pseudo-nidulans*, while Behrens had some strain of *A. glaucus*.

A. fumigatus var. *tumescens* Blumentritt, Ber. Deutsch. Bot. Ges. **23**: 419 427. pl. 19. figs. 5, 6, 18, 19, 20, 21. 1905. The culture described produced a dense, buckled, pseudo-parenchyma-like felt of mycelium with fruiting bodies not differing to any significant degree in measurements from *A. fumigatus*. Secondary heads from the outgrowth of sterigmata, branching stalks and septate stalks are figured. It is probably correctly characterized by the author a "culture cripple," since the differences are such as occur very commonly as a result of some unfavorable condition.

S. glauca Bainier, Bull. Soc. Bot. France **27**: 29. 1880. The description of this Sterigmatocystis is not complete enough to indicate its relationships.

A. glaucoides Spring, Bull. Acad. Sci. Belg. **19**: 560–572. 1852. The name without description was given to a colony which grew in an egg under experiment. The same form was afterward found in another egg. It is recorded as closely related to, if not identical with, the mold found in air sacs of birds, hence probably *A. fumigatus*.

A. gracilis Bainier, Bull. Soc. Myc. France **23**: 92. pl. 9. figs. 11–13. 1907. The description given by Bainier appears to relate *A. gracilis* to the *A. fumigatus* series.

A. gracilis var. *exiguus* Bainier & Sartory, Bull. Soc. Myc. France **28**: 47. pl. 2. 1912. According to the description this variety differs in physiological characters slightly from *A. gracilis* Bainier.

A. griseus Link, Sp. Pl. **6**: 69. 1824; Bonorden, Handb. Allg. Myk. p. 112. fig. 188. 1851. This was referred to by Wehmer (Monogr. p. 90) as probably *A. fumigatus*. Neither the description of Link nor the description and figure of Bonorden can be identified with certainty.

A. hageni Hallier, Cattaneo Mico. Corp. Um. p. 123. pl. 6. fig. 8.

Florentin. 1892; syn. *Otomyces Hageni* Hallier, Zeitschr. Parasit. 1: 195. 1869; and 2: 22, 233, and 259. pl. 5. 1870. In the latter article the descriptions are inadequate while the figures given include under *Otomyces hageni* fruiting hyphae which evidently represent Mucors, Penicillia, and probably at least 2 species of Aspergillus. This citation is included because most of the pathogenic Aspergilli seem to belong to the *A. fumigatus* or the *A. nidulans* series.

A. heterocephalus Spring, Bull. Acad. Sci. Belg. 19: 568. 1852. This name was given to colonies in a hen's egg which showed small heads globose and large heads columnar. Since no adequate figure or description was offered it may be discarded as a nomen nudum.

A. keratitis Ball, Amer. Med. 2: 31. 1901. This organism was found in an ulcer in the human cornea. No adequate description was given.

A. lignieresi Cost. & Lucet, Ann. Sci. Nat. IX. Bot. 2: 137. pl. 5. figs. 19–23. 1905. This culture from the lung of a penguin differs in cultural details from typical *A. fumigatus*, especially by the presence of swollen groups of cells in the mycelium.

A. malignus Lindt, Arch. Exp. Path. Pharm. 25: 256–271. figs. 1–11. 1889. While the description of this form is more or less incomplete and does not mention calyptriform heads, ascospore formation closely similar to that described by Lindt has been found by us in cultures with conidial fruits duplicating typical *A. fumigatus*.

A. microsporus Böke. The description and figures given by Cattaneo and Oliva in Arch. Lab. Bot. Critt. Garovaglio 5: 123. pl. 6. fig. 9. 1888, have been seen. No earlier or more complete description has been found. The organism was obtained from the human ear and has been listed as *A. fumigatus* but Wehmer (Monogr. p. 88) notes that the heads are figured as radiate, not calyptrate. The identity of Böke's form must remain doubtful.

S. minor Bainier, Bull. Soc. Bot. France 27: 30. 1880. The description as given is not sufficient to separate this from *A. nidulans*.

S. nidulans Eidam, Beitr. Biol. Pflanzen Cohn 3: 392–411. pl. 21, 22. 1879. A characteristic and cosmopolitan form discussed p. 96.

A. nidulans var. *Nicollei* Pinoy, Compt. Rend. Acad. Sci. Paris 144: 396. 1907. This variety was found fruiting within human tissue in a subject affected with "Madura-foot."

A. nigrescens Robin, Histoire Naturelle des Végétaux Parasites. p. 518. atlas. pl. 5. fig. 2. Paris. 1853. The organism of Robin has been called *A. niger* by Wilhelm (Beiträge zur Kenntnis der

Pilzgattung Aspergillus. pp. 70. Inaug. Diss. Strassburg-Berlin. 1877) and *A. fumigatus* by Siebenmann (Die Fadenpilze *Aspergillus flavus, niger* u. *fumigatus*; *Eurotium repens* (u. *Aspergillus glaucus*) und ihre Beziehungen zu Otomycosis Aspergillina. Inaug. Diss. Wiesbaden. 1883) and is judged undeterminable from the information given by Wehmer (Zur Kenntnis einiger Aspergillus-Arten. Centralbl. Bakt. II. **18**: 394–395. 3 fig. 1907.) Robin's figures represent *A. fumigatus* much more closely than *A. niger*.

A. Nölting Hallier, Zeitschr. Parasit. (not found) cited by Cattaneo and Oliva in Arch. Lab. Bot. Critt. Garovaglio **5**: 122. 1888. The conidia are described as yellowish. Other data are lacking but *A. flavus* alone of the organisms reported from the human ear commonly shows yellowish conidia.

S. olivacea van Tieghem, Bull. Soc. Bot. France **24**: 103. 1877. The data given are "common," "heads olive-green" and "on cochineal"; it must be dropped for lack of information.

A. olivaceus Preuss, Linnaea **25**: 77. 1852. Schroeter (Cohn, Krypt. Schles. 3^2: 216. 1893) notes that this description does not separate Preuss's material from *A. fumigatus* Fresenius.

A. penicilloides Spegazzini, Rev. Agrar. Veter. La Plata. p. 245. 1896. The description of this species might place it near to *A. fumigatus*. The organism was obtained from sugar-cane in Argentina. We have recently received a culture from Owen, in Louisiana, which probably represents the form described by Spegazzini but is not closely related to *A. fumigatus*.

S. prasina Bainier, Bull. Soc. Bot. France **27**: 31. 1880. This form is not recognizable from Bainier's description. It might have been a strain of *A. nidulans*.

S. pseudo-nidulans Vuillemin, Arch. Parasitologie **8**: 540–542. 1904. Vuillemin transfers the ascosporic form described by Grijns as *A. fumigatus* in Centralbl. Bakt. II. **11**: 330. 1903, to this specific name, amending Grijns's description by indicating the double nature of the band by which he separates his form *A. nidulans* as described by Eidam. The discussion by Vuillemin tallies with the commonest of our American soil forms of this group.

A. pusillus Massee, Kew Bull. Misc. Inf. **4**: 158. 1914. From the description this is a very small gray colony from soil in Africa, which would be readily distinguished from other members of the group. A relationship to the *A. fumigatus* series is possible.

A. quininae Heim. Bull. Soc. Myc. France **10**: 239. 1894. The

culture was found upon quinine solution but the description given will not separate it from *A. fumigatus*.

A. ramosus Hallier, Zeitschr. Parasit. 2: 266–269. pl. 6. figs. 1–6. 1870. The figures and descriptions evidently represent a strain of *A. fumigatus*.

A. rehmii Zukal, Oesterr. Bot. Zeitschr. 43: 160. pl. 11, 12. figs. 1–10. 1893. This species has been regarded as close to *A. nidulans* by some but Zukal's figures do not support that placing. The description given by Saito (Centralbl. Bakt. II. 17: 158) clearly places his organism in the *A. flavus* group.

A. roseus Batsch, Elench. Fung. 58; Fries, Syst. Myc. 3: 386. 1829. The only information given is "sporidiis roseis" with citations from Batsch, Sowerby, Persoon, Link, and Albertini and Schweinitz. The reference to this form is included because the colony color as in *A. terreus* approaches shades often designated as rosy.

A. roseus Link; Berkeley in J. E. Smith, Engl. Fl. 5: 340. 1836, cites *A. roseus* and attributes it to Link. Examination of Link, Sp. Pl. 6: 68. 1824, correctly carries the name back to Batsch (see preceding citation).

S. sydowi Bainier & Sartory, Ann. Mycol. 11: 25–29. pl. III. 1913. According to Herter (Myc. Centralbl. 3: 286–290. 1913), *S. sydowi* is a redescription of *A. nidulans*. The figures of *S. sydowi* do not justify this placing, however.

S. veneta Massalongo, Boll. Soc. Bot. Ital. 1900. p. 259. This form is described as forming yellowish hemispherical colonies upon rotten twigs; the fertile hyphae are fasciculate (form coremia?). Werkenthin (Fungous flora of Texas soils, Phytopathology 6: 247–249. 1916) identified certain cultures from Texas soil as *A. venetus* on account of fasciculate aerial hyphae. Cultures of these forms received from Werkenthin show that he had the form here described as *A. terreus*. We cannot agree with the identification of this form with *A. venetus* Massalongo.

A. virens Link, Obs. Ord. Pl. Nat. (Ges. Naturf. Freund. Berlin Mag. 3: 16. 1809); Sp. Pl. 1: 67. 1824. Saccardo F. ital. No. 20. Neither the description of Link nor the figure given by Saccardo suggests a relation to the calyptriform group.

A. virido-griseus Cost. & Lucet, Ann. Sci Nat. Bot. IX. 2: 140. 1905. The describers find this form to be pathogenic to rabbits not to fowls, and to be floccose whereas *A. fumigatus* is pathogenic also to fowls and is not floccose.

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A DEMONSTRATION OF PHOTOSYNTHESIS

W. J. V. OSTERHOUT

The difficulties in the way of a satisfactory demonstration of photosynthesis in land plants are too well known to require comment. They are greatest when quantitative results are desired. It is, of course, precisely these which are most important.

It is taken for granted that the reader is familiar with methods now in use.[1] The chief requisites appear to be (1) a method of removing at intervals satisfactory samples of the gases by which the leaf is surrounded (to accomplish this it is necessary to stir and mix the gases before taking the sample). (2) A method of gas analysis, simple and sufficiently accurate. (3) It is desirable to avoid the use of mercury, since the leaf is easily injured by mercury vapor.

The simple apparatus here described seems to meet these requirements and is easily constructed and kept in order. Its special advantage is that it permits the mixing of gases and the withdrawal of samples at will. In this way the progress of photosynthesis can be followed and the dynamics of the reaction investigated. It is also possible, when studying photosynthesis, to determine respiration without removing the leaf from the apparatus or changing the gases which surround it.

The apparatus consists of a wide-mouthed bottle (figure 1) or jar (the larger the better, up to a capacity of one gallon) with a stopper perforated by three or more short glass tubes (*R*). Each of these is connected (by rubber tubing) to a short glass tube (*S*) directly above it. This is in turn connected (by rubber tubing) to a tube about two feet long, (*A*), which is held in a vertical position by an arm (*T*) of a ring-stand. This tube should be of at least 9 mm.

[1] Cf. Ganong, W. F. Plant Physiology, 107 ff. 1908.

[The Journal for February (5: 55–104) was issued March 9, 1918.]

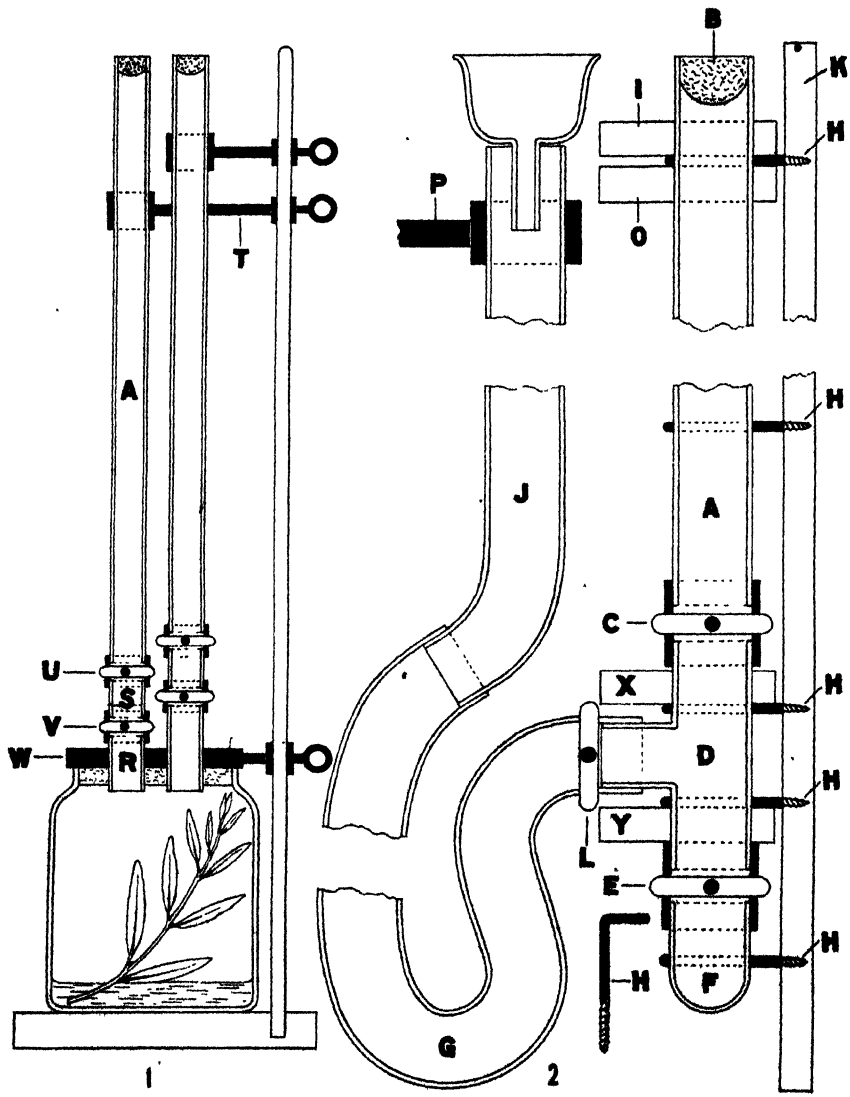


FIG. 1. Apparatus for the demonstration of photosynthesis. Samples of the gas (after thorough mixing by means of the water in the bottle) are removed in the analysis tubes (*A*).

FIG. 2. Apparatus for gas analysis. The gas is contained in *A*; *D* is filled with potassium hydrate and *F* with pyrogallol.

internal diameter. A ring (W) is fastened firmly against the stopper so that the stand may be inverted without displacing any part of the apparatus.

The procedure is as follows: We place vigorous plants[1] (covered with young leaves capable of active photosynthesis) in the bottle and fill it with water to about one tenth of its capacity. The stopper is then inserted. If provision has been made for three long tubes only one of them is now attached. One of the short tubes which perforate the stopper is connected to a CO_2-generator[2] (this tube is first clamped off). The other short tube is connected to a piece of glass tubing of small diameter.

The apparatus is now inverted, allowing the long tube to fill with water. Sufficient CO_2 is now admitted to drive out most of the water from the bottle through the narrow tube.[3] The apparatus is now returned to the upright position and the two remaining long tubes are connected to the bottle.

The apparatus is now inverted, allowing each of the long tubes to become partially filled with water. On placing in an upright position the water runs back into the bottle. This is repeated until the gases are well mixed and the water is nearly in equilibrium with them.

In case it is desirable to protect the leaf from wetting during the inversion of the apparatus the plant may be tied to a vertical support (passing downward through the center of the stopper) in such fashion that it does not touch the sides of the bottle.

By inverting the apparatus just enough water is allowed to run into one of the tubes, A, so that when in the upright position the water level can be seen above U. The tube A is then closed by two clamps, U and V. The apparatus is placed upright and the tube A is removed by cutting the rubber tubing below U or by slipping it off from S. This allows the removal of A without admitting air to A or to the bottle (the tube A being closed by the clamp U and the tube R by the clamp V).

[1] *Tradescantia* may be recommended for this purpose, especially kinds with non-striped leaves.

[2] The CO_2 must be well washed on its way to the bottle.

[3] Another procedure is to put into the bottle only enough water to fill two long tubes. Keeping the apparatus upright, run in CO_2 allowing it to displace air which issues through another short tube and is collected in an inverted graduate filled with water and dipping into water. When air representing about one tenth of the capacity of the bottle has been displaced by CO_2 the long tubes are put into place.

Care should be taken not to grasp the tube *A* directly, since this may warm the gas within it. It may be convenient to attach two spring clothes pins (one at either end) by which it may be handled.

The tube *A* is shown on a larger scale in figure 2: it is closed by a rubber stopper *B* (which may be shaped like a meniscus, as shown in the figure, though this is by no means necessary).

The tube is placed on the meter stick *K*, where it is held in place by "sash curtain hooks" *H*. These are pieces of metal bent at right angles, with a screw thread at one end. They are turned outward before the tube is in place. Then they are turned inward to hold it. The tube can be held in any desired position by attaching to it spring clothes pins (*I*, *O*) on each side of the sash curtain hooks. In consequence the tube is held firmly in place.

We now attach the rest of the apparatus. This consists of a T-tube *D*, a tube *F* (which is closed at one end), and a longer tube, *J*. All of these have a diameter as large as that of *A* (or larger). They are connected by two short pieces of rubber tubing furnished with clamps (at *C* and *E*) and a longer piece, *G* (with clamp at *L*). The tube *F* (which should be of the same diameter as *A* and at least 1 inch long) is filled with 20 percent aqueous pyrogallol[5] (taking care to exclude air), after which it is clamped off at *E* and the rubber connection *thoroughly* rinsed before being attached to *D* so that no pyrogallol can enter *D*.

The apparatus is placed on the meter stick and fastened by the hooks before being attached to the tube *A* (by means of the rubber tubing at *C*). It is very important that the tubes *A* and *D* be firmly attached to the meter stick so that they can not be pulled apart, allowing the reagents to escape.

A thistle tube is now placed in *J*, which is firmly fastened upon the retort stand by the clamp, *P*, so as to hang vertically. The meter stick with attachments is now inverted several times to make sure that the tubing is firmly attached to the meter stick and that all joints are secure. It is then hung on the retort stand in such a manner as to be easily detached. After opening the clamp at *L* we pour into the thistle tube 20 percent KOH until *D* is filled and the level appears

[5] This strength of pyrogallol will quickly (if above 15° C.) absorb at least 15 times its volume of oxygen. Hence if *F* is an inch long and filled with pyrogallol it can easily absorb at least 15 inches of oxygen in *A*. Ordinarily there are less than 8 inches of oxygen in *A*.

in *J*. The rubber tubing above *E* is squeezed to remove air. The meter stick is then inverted (without disturbing the tube *J*) and the rubber tubing at *C* is squeezed to remove air. When the air is all out of *D* the clamp at *L* is closed. The meter stick is returned to its upright position (as shown in the figure). The clamp *L* is then opened slightly so that when the rubber tubing above *E* is squeezed a movement of the liquid is seen in *J*. The clamp *L*, in this condition, permits the free movement of water while affording an effective barrier against the passage of a large gas-bubble.

The tubes are now adjusted so that the surface of the liquid is at the same level in *A* as in *J*. The position of the bottom of the stopper *B* is read off on the meter stick (the spring clothes pins, *O*, *I*, will hold it firmly in this position if properly adjusted). The clamp *C* is now opened somewhat, to secure atmospheric pressure in *A*, and the position of the meniscus at once read off on the meter stick (before lye has had time to diffuse into the tube above *C*). This gives the length of the gas column.

The clamp *C* is now fully opened and moved out of the way and the meter stick inverted (without disturbing the tube *J*). In doing this the operator should grasp the meter stick at the ends and avoid touching the glass tubing. If the glass tubing is handled the gas may be warmed by the contact and the tubes may be pulled apart at the joints (since the rubber tubing becomes slippery from the lye). There is some strain on the joints at *L* and at the end of *J*; it is very important that they be firmly secured by winding with wire or string (or they may be firmly held by means of spring clothes pins). As these are permanent connections it may be advisable to heat the glass before slipping the rubber tubing over it. On cooling the rubber adheres firmly to the glass. There is little or no strain on the other joints, but they should be secured as a matter of precaution.

When the meter stick is inverted the side neck of the T-tube should always point downward so as to prevent gas from entering it.

The rubber tube *G* should be kept in such a position that it never kinks in such a manner as to prevent free passage of liquid.

After inverting the meter stick several times in succession we restore it to the usual position and raise or lower the tube *A* until the liquid stands at the same level in *A* and in *J* (it may be necessary to pour more water into *J*). The length of the gas column in *A* is then read on the meter stick. The shrinkage, divided by the original length and multiplied by 100, gives the percent of CO_2.

After the reading is taken we invert the meter stick several times and take another reading. This should be continued until two successive readings agree.

We now open the clamp at *E*, allowing the pyrogallol to enter and absorb the oxygen. The procedure is the same as for CO_2. The shrinkage in the length of the column, due to absorption by pyrogallol, divided by the original length of the column and multiplied by 100 gives the percent of O_2.

If the apparatus were closed during the absorption by KOH and by pyrogallol there would be a tendency to suck in air at the joints. This is prevented by maintaining a passage for water through the clamp *L*, which is kept slightly open for this purpose.

It may be added that all the tubes (whether glass or rubber) must be large enough to permit gas to pass freely into them and displace water, and that *J* must be large enough to be easily filled with liquid through the thistle tube. An internal diameter of 10 mm. will be found sufficient.

In order to clean the apparatus it should be placed in running water under the tap and disconnected at *C* and *E*.

A slender tube of metal or glass should be connected to the tap and inserted to the very bottom of *A*, so as to rinse it thoroughly.

Students should practice analyzing the laboratory air for oxygen until the results are correct to within at least 2 percent. If the gas column is 600 mm. long 2 percent is 12 mm. on the tube. It is easy to read to 1 or 2 mm. (parallax must be avoided).

Since all the work is done at laboratory temperature (the reagents being at the same temperature) no correction is needed.

As we now know the composition of the gas at the start, we may expose the plant to sunshine (for several days in succession if desired) and again analyze the gas by removing another of the long tubes. If photosynthesis has not progressed satisfactorily another exposure may be made and the gas subsequently analyzed by removing another tube. After all the tubes are removed new tubes (filled with air or with water) may be attached, a correction being made for the air or water thus added to the system.

When the plant is exposed to sunshine the gas is heated and tends to escape at the joints: on cooling air may be sucked in. To prevent this (and to prevent diffusion of CO_2 through the rubber) all the rubber tubing should be coated with paraffin.

In addition a water seal may be provided by tying a piece of oil-cloth around the neck of the bottle so as to project in the form of a tube above it. This tube should be filled with water until all the joints are submerged. Or the whole apparatus may be placed in a large jar filled with water. These precautions will not be necessary if the joints are made secure by heating the glass before the rubber is slipped on, so as to make a permanent union on cooling.

It is important to have a control (containing no plants) which is kept beside the apparatus containing the plant. It is also desirable to have a control containing plants which is kept in the dark.

Respiration may be studied in precisely the same way as photosynthesis (it is not necessary in this case to add CO_2 at the start).

The method here outlined permits us to follow the progress of photosynthesis by making analyses at frequent intervals. In this way a time curve of photosynthesis may be plotted in order to study the dynamics of the process.

The influence of reagents (anesthetics, etc.) on respiration and photosynthesis may be studied advantageously by this method. It may be added that it has likewise been useful in studying the respiration of animals.

SUMMARY

An apparatus for the demonstration of photosynthesis is described which permits:

1. Removal at intervals of satisfactory samples of the gases by which the leaf is surrounded.
2. Stirring and mixing of the gases when necessary.
3. Analysis of the gases by a simple method which is sufficiently accurate for ordinary purposes.

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THE FLAVONES OF RHUS*

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Some time ago our interest in the possible formation of anthocyanins from flavones in plants led us to investigate *Rhus glabra* L., *R. typhina* L., and *R. copallina* L., all of them species with yellow wood, red fruits and exhibiting exceedingly brilliant red autumnal coloring. From various species of Rhus investigated by Perkin,[1, 2] flavones have been isolated and identified. No great difficulty was therefore anticipated in the study of the flavones, and these have been isolated and identified from *Rhus glabra* (leaves, both green and red, wood, and berries), *Rhus typhina* (wood), and *Rhus copallina* (green leaves). We have not been successful thus far in isolating the red pigments of the berries and autumn leaves, but the distribution of the flavones themselves seems sufficiently interesting to justify the publication of a note on the subject.

The genus Rhus, in the broad sense, includes several subdivisions which really seem to merit recognition as genera. The type species of Rhus in the restricted sense is, as Greene[3] has shown, *Rhus Coriaria* L., the Sicilian sumach. With this species, to which on account of its commercial importance most of the chemical investigators have directed their attention, the three species studied by us are congeneric. One of them, *Rhus glabra*, is a collective species, including a number of elementary species, but their limits are not well known, and for the purpose of this paper it will suffice to use the name *R. glabra*, with the qualification that the data apply only to a form occurring about Ann Arbor, Michigan.

* The work here reported was carried on in the laboratories of the office of fermentation and physiological investigations, Bureau of Plant Industry, and the Department of Botany of the University of Michigan. Published by permission of the Secretary of Agriculture.

[1] Perkin, A. G., and Allen, G. Y. Colouring matter of Sicilian sumach, *Rhus Coriaria*. Journ. Chem. Soc. (London), Trans. 69: 1299–1303. 1896.

[2] Perkin, A. G. Yellow colouring principles contained in various tannin matters. Part VI. *Rhus Cotinus* and *Rhus Rhodanthema*. Journ. Chem. Soc. (London), Trans. 73: 1016–1019. 1898.

[3] Greene, E. L. A study of *Rhus glabra*. Proc. Washington Acad. Sci. 8: 167–196. 1906.

Perkin has isolated flavones from *Rhus Coriaria*,[4] *R. Metopium*[4] (= *Metopium Metopium* (L.) Small), *R. Cotinus*[2] (= *Cotinus Coggyria* Scop.), and *R. rhodanthema* F. v. M.[2] (= *Rhodosphaera rhodanthema* (F. v. M.) Engl.). Only in the last two does the wood contain a flavone, and in both cases it is fisetin. In the leaves of the first two, the leaf flavone is myricetin, in the third both myricetin and quercetin occur, and in the last quercetin[2] alone. Perkin[1] shows definitely that early workers who ascribed quercetin (either free or glucosidal) to the leaves of *R. Coriaria* and *R. Cotinus* were in error. The data given above provide our only reliable data in regard to the distribution of the flavones in Rhus. Perkin emphasizes the fact that in all species thus far investigated the wood flavone is different from the leaf flavone. Our paper provides further data for establishing a generalization with regard to this point, and also gives the first identifications of flavones from American sumachs.

Acree and Syme[5] have reported fisetin from the leaves of *Rhus Toxicodendron* L. (= *Toxicodendron vulgare* Mill.), in which they suppose it to occur both free and as one constituent of a glucoside to which they ascribe the poisonous properties of this plant. They give no analysis of the flavone which they isolated, but from their own statement that it yields protocatechuic acid and phloroglucinol on potash fusion, we infer that it was quercetin rather than fisetin. Fisetin would have given protocatechuic acid and resorcinol. In a recent refutation of a paper by McNair[6] in which the work of Acree and Syme is called into question, Acree[5] explicitly verifies the earlier statement of Acree and Syme that a quantity (two grams) of the flavone which they obtained from the leaves of *R. Toxicodendron* and identified as fisetin was decomposed by potash fusion into phloroglucinol and protocatechuic acid. He states that the color reactions of the flavone were those of fisetin, but unless these substances are very carefully purified, there is much likelihood of error. Quercetin or even luteolin

[4] Perkin, A. G. Yellow colouring principles contained in various tannin matters. Part VII *Arctostaphylos Uva-ursi, Haematoxylon Campeachianum, Rhus Metopium, Myrica Gale, Coriaria myrtifolia*, and *Robinia pseudacacia*. Journ. Chem. Soc. (London), Trans. **77**: 423–432. 1900.

[5] Acree, S. F., and Syme, W. A. Some constituents of the poison-ivy plant. Amer. Chem. Journ. **36**: 301–321. 1906. The same paper under a different title. Journ. Biol. Chem. **2**: 547–573. 1906–7.

[6] McNair, J. B. The poisonous principle of poison oak. Journ. Amer. Chem. Soc. **38**: 1417–1421. 1916.

might have been confused with fisetin, and it is extremely likely that quercetin was the flavone which they obtained. Not only is it more widely distributed than luteolin, but it is already known from the leaves of *Rhus rhodanthema*.[2] Even on the supposition that Acree and Syme incorrectly identified the potash fusion products of their flavone, it is still unlikely that they had fisetin, since the evidence now at hand seems to show that the latter does not occur in leaves, even when present in wood of the same plant. In their papers Acree and Syme refer to early work of Schmid[7] who did indeed state (erroneously) that one of the products resulting from the potash fusion of fisetin was phloroglucinol. His work preceded the determination of the constitution of fisetin, and has been superseded.

McNair made gasoline extractions of *Rhus diversiloba* T. and G. (a species closely allied to *R. Toxicodendron*) and failed to find any flavone at all in the extracts. This result is, of course, what one would expect, since both the free flavones and their glucosides are insoluble in gasoline. McNair makes the point, which seems to be well taken, that since the poison of the poison-ivy is soluble in gasoline, whereas the flavone glucosides are not, Acree and Syme cannot have been correct in ascribing the poisonous qualities of the plant to such a glucoside. On the contrary, his negative work obviously has no bearing on the question of what flavones occur in the poison-ivy.

The Wood Pigment, Fisetin

The wood of *R. typhina* was collected near Washington, D. C. A single log, four feet long and about seven inches in diameter, afforded all the material used. The wood of *R. glabra* was all collected from a single thicket near Ann Arbor, Michigan. The maximum diameter of the sticks was about 2 inches. In the case of each species the method of extracting the flavone, which proved to be fisetin, was as follows. The white sap wood was removed, and the yellow heart wood reduced to small chips, which were boiled for several days with successive portions of distilled water. The decoctions were combined, evaporated to small bulk, filtered, and shaken out with ether. The impure ethereal solution of fisetin thus obtained was evaporated and the residue extracted repeatedly with water, in which fisetin is practically insoluble, whereas certain colored impurities are soluble. It was then

[7] Schmid, Jakob. Ueber das Fisetin, den Farbstoff des Fisetholzes. Ber. Deutsch. Chem. Ges. 19: 1734–1749. 1886.

dissolved in a small volume of hot alcohol, filtered, and fractionally precipitated with water. The fractions were separately dried and acetylated by heating for an hour with anhydrous sodium acetate and acetic anhydride. The reaction mixture was poured into water and after twenty-four hours the precipitated acetyl fisetin was filtered off and purified by recrystallization from alcohol. The acetyl derivative formed a mat of silk-like colorless needles, insoluble in water, insoluble in cold, and sparingly soluble in hot alcohol, and easily soluble in warm glacial acetic acid. Since the several fractions had the same melting point, 190–200.5° C. (uncorrected), they were combined and again purified by recrystallization. The purified acetyl fisetin, derived from *R. typhina*, gave a yield of 61.66 percent of fisetin by hydrolysis, agreeing satisfactorily with the theoretical yield of 62.99 percent from $C_{15}H_6O_6(C_2H_3O)_4$. Had the formula of the acetyl compound been $C_{15}H_5O_6(C_2H_3O)_5$ theory would have required 57.66 percent flavone; if $C_{15}H_7O_6(C_2H_3O)_3$, 69.41 percent. The hydrolysis was carried out in acetic acid solution, with sulphuric acid. The recovered flavone was precipitated by the addition of water. The acetyl fisetin derived from both species had the same melting point, and since combustions of the recovered flavone were made for both, it was deemed sufficient to make combustions of the acetyl fisetin from one source only. The results are given in Table I.

TABLE I

Combustions of Acetyl Derivative of Fisetin from Wood of Rhus typhina

Sample	Weight	CO_2	H_2O	C. %	H. %	O. %
A	.1225	.2724	.0418	60.64	3.82	35.54
B	.1054	.2372	.0382	61.37	4.06	34.57
C	.2046	.4600	.0724	61.31	3.96	34.73
Arithmetic mean of three determinations		...	...	61.10	3.94	34.96
Weighted mean				61.14	3.95	34.91
Required for $C_{15}H_6O_6(C_2H_3O)_4$				60.79	3.96	35.25

The pure fisetin was of a pale lemon yellow color, insoluble in cold water, very sparingly soluble in hot water, readily in alcohol and acetone. It was removed from aqueous or very dilute alcoholic solutions by acetic ether or ether, but after drying dissolved in ether only with difficulty. It was insoluble in benzene and chloroform. With ferric chloride it gave an olive-green coloration, with lead acetate an orange-red precipitate, with ammonia and other alkalies an intensification of

the yellow color. The data for the combustions of fisetin follow in Table II.

TABLE II

Combustions of Fisetin, Recovered from Acetyl Fisetin

Sample *A* from wood of *Rhus typhina*, sample *B* from wood of *R. glabra*.

Sample	Weight	CO_2	H_2O	C,%	H,%	O,%
A	.1074	.2482	.0350	63.02	3.64	33.34
B	.2550	.5870	.0796	62.78	3.49	33 73
Required for fisetin, $C_{15}H_{10}O_6$				62.94	3.49	33.57

The cleavage products of fisetin were determined in the usual way by potash fusion. The material was heated thirty minutes at 170–200° C. with potassium hydroxide and a very small amount of water. The melt was dissolved in water, neutralized with hydrochloric acid, and shaken with ether. The residue after evaporating the etherial solution was neutralized with sodium bicarbonate and again shaken with ether.

The ethereal layer contained resorcinol, identified as uch after purification by sublimation between watch glasses. It was easily soluble in water, gave a violet color with ferric chloride, and melted at 106–108° C. (uncorrected). Rosenthaler (Der Nachweis organischer Verbindungen) accepts the value 110–111°, but quotes 118° as E. Schmidt's determination of the melting point of the absolutely pure compound. In some of the text-books (*e. g.*, Richter) the melting point is given as 118°. Landolt-Börnstein gives it as 111.6°, and this value is undoubtedly correct, being taken from a recent determination (1911) of Timmermans. Perkin and Gunnell[8] found that a Kahlbaum preparation melted at 108–109° C. With this value ours is in excellent agreement.

The sodium carbonate solution, after the removal of the resorcinol, was acidified and shaken out with ether. The latter removed a substance shown to be protocatechuic acid. It decomposed on heating, yielding a sublimate of catechol, identified as such by its melting point and reaction with ferric chloride (a green color, passing to violet, then red, upon addition of sodium bicarbonate).

There can be no doubt, therefore, that fisetin is the wood flavone of both *Rhus typhina* and *R. glabra*.

[8] Perkin, A. G., and Gunnell, O. The colouring matter of Quebracho Colorado. Journ. Chem. Soc. (London), Trans. **69**: 1303–1309. 1896.

The Leaf Pigment, Myricetin

Several variations in method were used for the isolation of myricetin from the leaves. The best yield was obtained by the method of Perkin. This method consists in fractionally precipitating the dissolved substances from an aqueous extract of the leaves, with lead acetate. On the addition of this reagent impurities (tannins, gums, resins, etc.) are first precipitated as lead compounds and may be removed by filtration. The flavones and their glucosides are only precipitated upon the addition of an excess of lead acetate.

The dried leaf powder was treated several days with successive portions of boiling distilled water and the combined extracts evaporated to small bulk. Lead acetate was then cautiously added to the boiling mixture until a further quantity produced a yellowish precipitate. In this manner the impurities were got rid of, by filtering off the lead compounds first precipitated. Excess of lead acetate added to the filtrate produced an insoluble yellow lead salt of the flavone glucoside. This was filtered with suction, washed thoroughly with water and decomposed with boiling dilute sulphuric acid. Lead sulphate was filtered off, and the filtrate, when cold, shaken with ether. The residue after evaporation of the ether contained the flavone and gallic acid. The latter was removed by treatment with hot water. The flavone was filtered off, dried, and acetylated in the usual manner.

The other method that we found useful in the isolation of myricetin was as follows. The aqueous extract of the leaves was treated with a large amount of hide powder to remove tannin. The filtrate was then evaporated to small bulk and hydrolyzed with hydrochloric acid (33 percent by volume) for nearly an hour. An ether extract of the cold solution yielded the crude pigment, which was purified by the usual process of acetylization and recovery by hydrolysis.

Our yields of myricetin were very small, and insufficient for combustions to be made of the compound from all of the sources from which it was obtained. It was likewise impossible to make a potash fusion. Myricetin, however, has more characteristic qualitative reactions[9] than the other pigments of the flavone group. Ammonia and dilute alkalies give a green coloration, changing to blue, violet, and finally reddish-brown. No other known flavone gives this play

[9] Perkin, A. G., and Hummel, J. J. The colouring principle contained in the bark of *Myrica nagi*. Part I. Journ. Chem. Soc. (London), Trans. 69: 1287–1294. 1896.

of colors. Ferric chloride gives a brownish-black color. The myricetin is darker in color than fisetin, and shows about the same solubilities; it differs in being slightly soluble in chloroform and only very slightly in acetic acid.

The largest sample of myricetin was obtained from leaves of *Rhus glabra*. The acetyl myricetin from this source yielded 55.00 percent of myricetin on hydrolysis. Theory requires 55.79 percent for $C_{15}H_4O_8(C_2H_3O)_6$, the formula of the compound. For $C_{15}H_5O_8(C_2H_3O)_5$ and $C_{15}H_3O_8(C_2H_3O)_7$ the yields would have been 60.22 percent and 51.96 percent respectively. Two combustions of the acetyl myricetin and one of myricetin were carried out, with results shown in Table III. The acetyl myricetin melted at 208–209° C. (uncorrected) when slowly heated. Perkin gives the melting point as 211–212° C.

TABLE III

Combustions of Myricetin from Green Leaves of Rhus glabra (Sample A) and of its Acetyl Derivative (Samples B and C)

Sample	Weight	CO_2	H_2O	C%	H%	O%
A	.0800	.1656	.0290	56.45	4.06	39.49
Required for myricetin, $C_{15}H_{10}O_8$				56.60	3.14	40.26
B	.0960	.2012	.0360	57.15	4.20	38.65
C	.1332	.2784	.0510	57.01	4.27	38.72
Mean of two determinations				57.08	4.24	38.68
Required for acetyl myricetin, $C_{15}H_4O_8(C_2H_3O)_6$				56.84	3.86	39.20

Myricetin both glucosidal, and, in very slight traces, free, was also obtained from the red autumn leaves of *R. glabra*, and from the red berries. In the latter case the myricetin was free, but it may have been derived by hydrolysis from a glucoside, since the berries when boiled in water yield a strongly acid solution. Two samples of green leaves of *Rhus copallina*, kindly furnished by Dr. W. W. Stockberger, of the Bureau of Plant Industry, also proved to contain myricetin. They were collected by C. R. Gilmore at Muskogee, Oklahoma. Several attempts to isolate a flavone from the leaves of *R. typhina*, collected at Ann Arbor, were unsuccessful, although various methods were used and the operations were conducted on a large scale.

SUMMARY

By the isolation of flavone pigments from three species of Rhus, *R. typhina*, *R. glabra*, and *R. copallina*, we have been able to verify

Perkin's conclusion that the same flavone is not likely to be found in both the wood and leaves of the same species. Fisetin is distinctively a wood flavone, and would appear to be an end product of metabolism. It is now known from *Rhus Cotinus*, *R. rhodanthema*, *R. typhina*, and *R. glabra*. The first two do not belong to Rhus in the restricted sense, but to the genera Cotinus and Rhodosphaera, respectively. Our studies are therefore the first to demonstrate the presence of fisetin in wood of species belonging to Rhus proper (the true sumachs).

The distinctive leaf flavone of Rhus proper is myricetin. It has been known from *R. Coriaria*, and we are able to add *R. glabra* and *R. copallina*. It is probably a plastic substance. Although we have thus far been unable to trace its relationship to the fisetin of the stem, or to the anthocyanins of the leaf and berries, efforts along this line will not be abandoned. The flavones are becoming increasingly interesting to the physiologist and geneticist, and on this account we venture to present this slight addition to our knowledge of their distribution in plants.

CONSERVATISM AND VARIABILITY IN THE SEEDLING OF DICOTYLEDONS

EDMUND W. SINNOTT

INTRODUCTION

Ever since the statement of the law of recapitulation by the zoologists attempts have been made to extend it to the plant kingdom and to discover, in the seedling, traces of characters which have been lost elsewhere in the plant and which from their constancy might be used as guides to relationship. External characters, particularly "juvenile" foliage, were at first chiefly studied, but more attention has latterly been paid to internal structure. Jeffrey and his students have investigated the secondary wood of young plants for evidences of conservatism. It is the structure of the actual seedling, however, and its still dominant primary tissues, which has engaged the attention of most students of the subject, foremost among whom are members of the English school of anatomists.

Although numerous instances have been found by these observers where seedling structure is useful for classification, within narrow limits, it must be admitted that the high degree of variability recorded in "type of symmetry," number of protoxylem clusters, number and position of primary bundles, level of transition from root structure to stem structure, and so on, have served in the minds of many to cast doubt on the conservatism of this portion of the plant and have discouraged those who attempt to promulgate the law of recapitulation for the vegetable kingdom. The opinion of the probably majority of workers is expressed by Hill and de Fraine in the following quotation (1, p. 264): "For these reasons we see no necessity for preserving seedling anatomy from the fate already meted out to other structural features, *e. g.*, secondary thickening, which were at one time considered as indicators of phylogeny, a conclusion arrived at, either entirely or in part, by others who have paid attention to the facts of seedling anatomy."

These investigators have in general confined themselves to one or a few families and have studied the relative variability of the

seedling and the mature portions of the plant, looking to the former to provide clues for specific or generic relationship. The present paper records an examination of seedling structure over a wide range of families and orders in the dicotyledons, with a view to determining, on the basis of this comparative study, whether the anatomy of the seedling is variable everywhere, or whether it exhibits any characters which are sufficiently constant to be of value in marking out broad lines of relationship.

The seedlings of over 250 species belonging to 86 families were examined. External characters were observed and recorded and internal structure was studied by means of serial sections.

Observations

The observations of others as to the high degree of variability of certain characters, particularly the number of protoxylem clusters and the level of transition, were confirmed and their uselessness for classification emphasized. Another line of inquiry, however, was

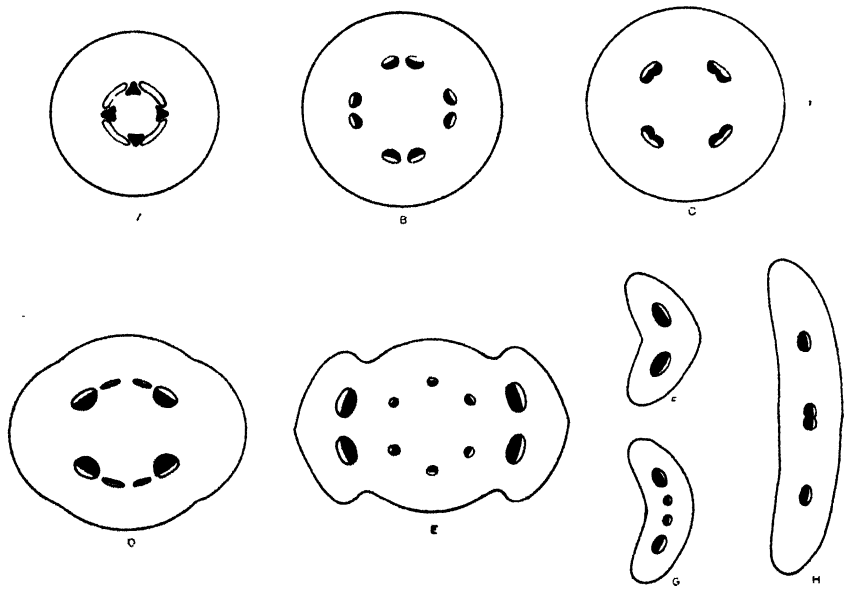


FIG. 1. *Thespesia populnea* (Malvaceae). Serial sections from root to cotyledon. *a*, root; *b–d*, hypocotyl; *e*, node (bundles of epicotyl in central ring); *f–g*, petiole of cotyledon; *h*, base of cotyledonary blade. (Xylem black, phloem white.)

suggested by the fact that in the mature plant the topography of the node, with the number and arrangement of leaf traces and gaps, has been shown (2) to be very constant throughout wide groups. To determine whether or not the characters of the cotyledonary node

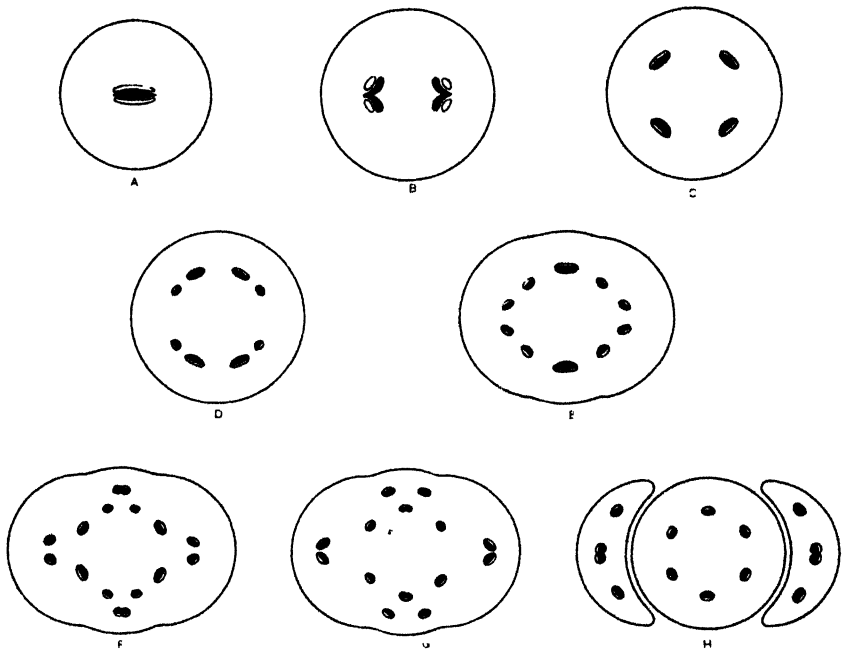


FIG. 2. *Momordica Balsamina* (Cucurbitaceae). Serial sections from root to cotyledon. *a*, root; *b–d*, hypocotyl; *e g*, node (showing relation of cotyledonary bundles to those of epicotyl); *h*, epicotyl and cotyledons. (Xylem black, phloem white.)

are also slow to change, careful attention was given not only to the vascular supply of the seedling proper, consisting of the cotyledonary traces and their extensions into the hypocotyl, but to the relation between these first strands and those which arise later and form the vascular system of the epicotyl and subsequently the stem of the young plant. It was found that this relationship between the hypocotyledonary and the epicotyledonary systems, a feature hitherto neglected, provides some of the most constant structural characters of the seedling.

By far the most common condition at the cotyledonary node is that shown in figures 1 and 2 and in figure 4, *b*, *c*, and *d*, where the bundles of the epicotyl arise entirely in the intercotyledonary plane and the traces of each cotyledon make but a single gap in the vascular ring. This corresponds to the unilacunar nodal type in the mature

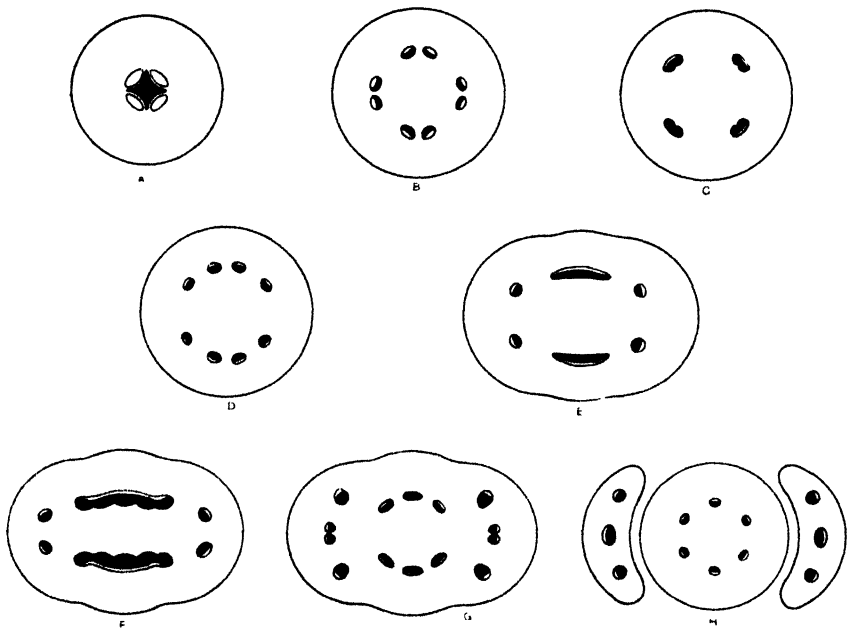


FIG. 3. *Echinops sphaerocephalus* (Compositae). Serial sections from root to cotyledon. *a*, root; *b–e*, hypocotyl; *f–g*, node (showing trilacunar insertion of cotyledonary traces); *h*, epicotyl and cotyledons. (Xylem black, phloem white.)

stem. A number of families, however, show a more complex condition, the epicotyledonary bundles arising between the individual strands of a cotyledonary trace. In figures 3 and 4, *c*, the trace has four strands and the epicotyledonary bundles arise between each lateral and its adjacent central bundle. The vascular supply of each cotyledon thus causes three gaps in the ring (in this case two lateral traces arise from the same gap) and thus corresponds to the trilacunar condition of the mature stem. Several variations on this type were observed. In some cases, bundles of the epicotyl appear at the inter-

cotyledonary poles, separating the adjacent laterals. Sometimes, as in Ricinus, epicotyledonary bundles may also appear between the two central strands of the trace, thus causing each trace to leave four gaps in the vascular ring. Sometimes, as in certain of the Euphorbiaceae, Aceraceae and Proteaceae (Fig. 4, *f*), the cotyledonary trace

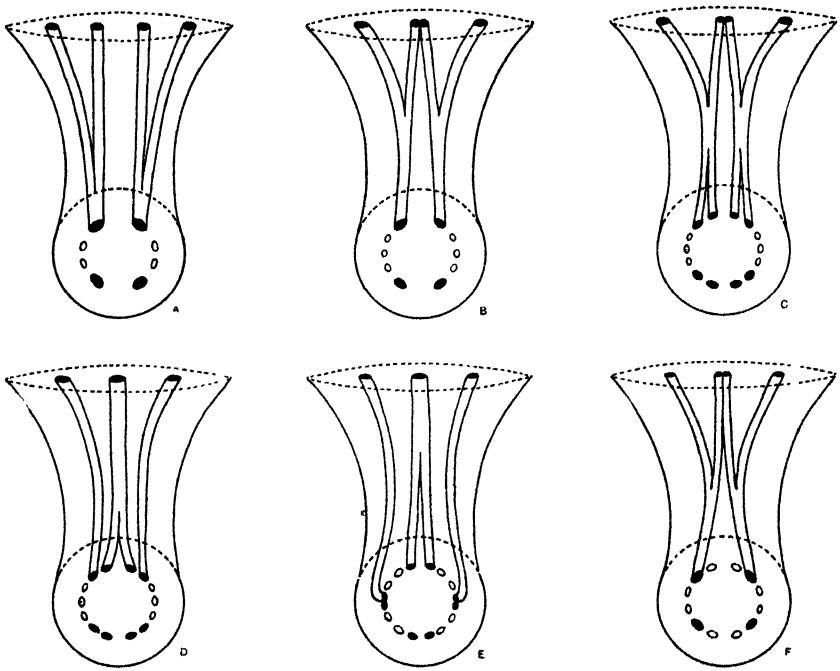


FIG. 4. Diagrams showing cross-sections of node, course of bundles in base of one cotyledon, and section of cotyledon, in six seedling types. *a*, *Ephedra dioica*, gymnospermous type, unilacunar, two trace bundles, no midrib; *b*, *Thespesia populnea*, unilacunar, two trace bundles, giving rise to three-veined condition; *c*, *Lavatera arborea*, unilacunar, four trace bundles fusing into two and then producing three-veined cotyledon; *d*, *Momordica Balsamina*, unilacunar, four trace bundles forming three-veined condition directly; *e*, *Echinops sphaerocephalus*, trilacunar, four trace bundles, forming three-veined cotyledon; *f*, *Grevillea robusta*, bilacunar, two trace bundles, forming three-veined cotyledon. (Leaf traces black in section, epicotyl bundles white.)

may consist of only two strands, but these may be separated by epicotyledonary bundles, a bilacunar node thus being produced. In all these cases the essential fact is that the node is multilacunar, the

vascular tissue of the epicotyl arising somewhere between separate strands of the same trace. This condition, it should be noted, is not simply that of "independent laterals," which may occur in either of the main forms we have described.

These two types of cotyledonary node are very constant through large groups. The unilacunar is much the more common and is invariably present (as far as the writer has observed) in 73 out of the 86 families examined. The multilacunar type was found in the Aceraceae, Berberidaceae, Compositae, Euphorbiaceae, Hippocastanaceae, Magnoliaceae, Melianthaceae, Plumbaginaceae, Polygonaceae, Proteaceae, Sapindaceae, Sapotaceae and Umbelliferae. In the Berberidaceae (Berberis) the two lateral strands of the trace are very small and are frequently absent, giving a unilacunar condition. In the Magnoliaceae several genera are unilacunar, as is Securineca in the Euphorbiaceae. All the other genera and species of these and the other families named were invariably found to be multilacunar, of one type or another. In the Aceraceae, Sapotaceae and portions of other families there are two gaps at the node; in the others, prevailingly three.

Since the size of the seedling has been shown by Hill and de Fraine (1) to affect the number of protoxylem clusters in the root and hypocotyl and the number of strands in the cotyledonary trace (the larger the seedling, the greater the number) it might also be expected to affect the topography of the node. In very tiny seedlings it may do so, but in no case observed was this found to be true. Some very small seedlings were studied in the multilacunar families, particularly in the Compositae and Umbelliferae, but these were always multilacunar. Many of the largest seedlings, on the other hand (notably the Leguminosae as a whole), are unilacunar.

There is an evident relation between the structure of the node in the main stem and that in the seedling. All families in which the seedling shows a multilacunar condition possess this type in the mature stem also, except in the case of the Sapotaceae. In the 73 families where the cotyledonary node is unilacunar, however, the connection is less definite, for 34, or 47 percent, have a prevailingly unilacunar node in the mature plant; 39, or 53 percent, being multilacunar.

These facts indicate that in the topography of the cotyledonary node we have a character which is much more constant than many of

the anatomical features of the seedling, and that it may be used to distinguish large groups of plants. Of course the number of species studied is far too small to give an accurate idea of what the conditions are throughout the various families, but in those where the largest number of species has been recorded, nodal uniformity is very evident. In several cases both main types are found in the same family. The Ranunculaceae are prevailingly unilacunar, but certain species of Clematis seem from their description by Miss Thomas (5, p. 706) to have several gaps. We have recorded a similar situation in Berberis, the Magnoliaceae and the Euphorbiaceae. It seems very likely that in many of these less specialized families the seedling node may not be completely uniform. As to its general conservatism throughout the dicotyledons, however, there can be little doubt.

A comparative study of the seedling node will evidently give us much valuable information as to relationships. The multilacunar condition seems to be quite absent in many of the great orders, even in those where the node of the mature stem has several gaps. In others it is invariably present in all species so far examined. In still others, notably the large and heterogeneous orders Geraniales and Sapindales, it characterizes certain families or groups of families but is absent from the rest, thus providing a clue as to relationships within the order. In the Sapindales, for example, the Aceraceae, Hippocastanaceae and Sapindaceae, grouped together by Engler as the sub-order Sapindineae; and the Melianthaceae, comprising the sub-order Melianthineae, are multilacunar. All the rest of the families examined in this order, however, are unilacunar.

As to what has been the evolutionary history of the seedling node we cannot be sure. Evidence has elsewhere been presented (2) that for the mature stem the trilacunar node was the primitive one among Angiosperms. The fact that this is present in the seedling node of only a few families, however, and that these are for the most part by no means primitive in their other characters, suggests that the unilacunar condition of the seedling is a persistence of an ancient gymnospermous condition and that the multilacunar type made its appearance in the node of the foliage leaf and has worked down from thence into the seedling. On the contrary, it may be argued from those cases where the lateral traces in the multilacunar condition are very small, that they are here dying out; and that the unilacunar type has arisen by the complete loss of lateral traces which originally were always present.

A second feature of the structure of the seedling which is constant throughout large groups is the venation of the cotyledon. In the great majority of the families this is palmate and three-veined (3, Plate III), a condition which we have reason to believe is primitive for the Angiosperms. In a few cases, however, it seems to be constantly pinnate, with a strong midrib. This is characteristic, so far as the writer's observations have gone, of the Amaranthaceae, Anacardiaceae, Boraginaceae, Capparidaceae, Celastraceae, Ebenaceae, Moraceae, Myoporaceae, Nyssaceae, Periplocaceae, Pittosporaceae, Polemoniaceae, Rutaceae, Simarubaceae, Solanaceae, and of portions of other families. In all these cases the cotyledonary node is unilacunar, so that there is in the seedling a similar relation between nodal topography and leaf venation which has been found to occur in the mature plant (3).

The most constant and invariable character of the seedling, however, is the double nature of the cotyledonary trace, a fact emphasized by the work of Miss Thomas (4). In the ferns and gymnosperms the trace of the mature leaf, where it leaves the vascular ring, is at least primitively either a double bundle or an arc with an even number of bundles. A radical change brought about at the origin of the Angiosperms was the conversion of this double bundle into a single one or into an arc with an odd number of strands. This change is made evident externally by the development of the strong midrib so characteristic of the leaf of Dicotyledons. In the seedling of the Dicotyledons, however, we find the original condition persisting. The venation of the cotyledon, to be sure, is angiospermous, with a midrib and lateral veins, which distinguish it from the cotyledons of the gymnosperms.[1] At the cotyledonary node, however, the ancient double trace still persists unchanged. *In its essential topography the node of the seedling is the same throughout all seed plants.* In figure 4, *a*, is shown the node of Ephedra, presenting the typical gymnospermous condition, and in the other figures are some of the types found among dicotyledons. There may simply be two traces to each cotyledon or there may be two pairs of traces. In the trilacunar type it will be noted that an even number of bundles is given off, due to the fact that the central gap provides two strands.

It is usually in the petiole of the cotyledon that the transition

[1] In many conifers, where the seed-leaves are numerous and needle-like, a single bundle is sometimes all they possess.

from this ancient even-bundled type to the odd-bundled angiospermous condition of the blade takes place by the fusion of the two central bundles or branches to form a midrib (Fig. 4, *b*, *c*, *d*, *e*, *f*). This may occur in various ways and at various levels, but the result is always the production of a midribbed cotyledon unlike that characteristic of gymnosperms. The two bundles of which the midrib is composed often do not fuse, but run close together through the blade and diverge again widely near the tip of the cotyledon.

Discussion

It is therefore evident, from a study of only a few of its features, that the structure of the seedling displays various categories of characters differing in the amount of conservatism which they possess. The number of protoxylem groups and the level of transition (together with many other characters) vary from species to species and from genus to genus; the number of main veins in the cotyledon (whether one, producing a pinnate blade, or three, five or more, forming a palmate one), and the method of insertion of the cotyledonary traces, are much more constant and distinguish families or groups of families; the main type of venation of the cotyledon (whether midribbed or dichotomous) is still more constant and serves to distinguish angiosperms from gymnosperms; and finally the type of cotyledonary trace (the double bundle and its modifications) is essentially uniform throughout all seed plants.

No general statement that the seedling as a whole is "conservative" or "variable" can therefore well be made. Certain of its characters are highly conservative and certain others are highly variable, the emphasis placed on the one group or the other having led to the differences of opinion as to the general conservatism of the seedling. Indeed, it should be recognized that throughout the plant body it is not particular organs or regions which are less variable than others, but particular *characters of the plant.* In certain structures, especially the flower, these conservative characters are very noticeable, as in the relations of coalescence, adnation and number. Such a large number of other floral characters are highly variable, however, that it is obviously impossible to regard the flower *as a whole* as conservative. So many conservative characters have recently been found in different parts of the plant as to suggest that when our knowledge of comparative plant anatomy is more complete, we shall find that no

one region possesses a very much larger proportion of these slowly changing features than does any other. Of course we must recognize that a given character may be variable in one family or group and conservative in another. This emphasis on the single character, however, rather than on the whole structure, in a consideration of variability, is clearly in harmony with modern conceptions of heredity.

The very fact that there are such things as "conservative characters," which for some reason have become so firmly fixed in the germ-plasm that they have been consistently less variable than others throughout large groups of organisms during evolutionary history, is, of course, what makes it possible for us to recognize relationship and to construct a "natural system" of classification. A clear demonstration of this principle is one of the chief contributions of phylogeny to those sciences which are concerned with the method of evolution.

SUMMARY

1. The present paper is a comparative study of the structure of the seedling throughout the Dicotyledons, with a view to determining the degree of conservatism which it exhibits.

2. Certain features, notably the number of protoxylem clusters and the level of transition from root to stem structure, were found to be very variable, thus confirming the results of other investigators.

3. Of much more constancy was found to be the relation between the vascular system of the hypocotyl and that of the epicotyl. Two main types were recognized; that in which the cotyledonary trace makes but a single gap in the epicotyledonary system, and that in which more than one gap (usually two, three or four) is produced.

4. The venation of the cotyledon, whether of three main palmate veins or of a single strong midrib with weak side veins, was found to be very constant.

5. An *odd* number of veins in the cotyledon (a midrib, usually with one or more pairs of main lateral veins) was found to characterize the seedling of all dicotyledons, and to distinguish it from that of gymnosperms with broad cotyledons.

6. The most conservative character in the anatomy of the seedling is the structure of the cotyledonary trace, which throughout dicotyledons is a double bundle or a modification of it, a type universal among seed plants and also, at least primitively, in the foliage leaf of ferns and gymnosperms.

7. The seedling of dicotyledons is therefore variable in certain of its characters and conservative in others, thus emphasizing the importance of studying conservatism and variability in connection with particular characters rather than with particular organs or regions.

The writer wishes to thank Professor I. W. Bailey, of the Bussey Institution of Harvard University, for the privilege of looking over many of his preparations and for information as to seedling structure in a number of families.

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NOTEWORTHY LEJEUNEAE FROM FLORIDA[1]

ALEXANDER W. EVANS

Our knowledge of the Hepaticae occurring in Florida has been materially increased during the past few years. This is due in great part to the careful collections made by Mr. Severin Rapp in the vicinity of Sanford, Orange County, although Dr. J. K. Small, Mr. N. L. T. Nelson and other collectors have made notable discoveries. In 1915 Miss Caroline C. Haynes[2] published a list of sixty-four species which Mr. Rapp had found, including twenty-four members of the Lejeuneae. In the present paper six additional Lejeuneae are noted. Four of these are apparently undescribed, although one has already been reported from Sanford under another name. One of the remaining species has been previously reported from Cuba and the other from Jamaica. Of the new species two, according to our present knowledge, are endemic to Florida. The number of Lejeuneae now known from Sanford is twenty-nine; from the entire state of Florida, forty-four; from the entire United States, forty-eight.

1. **Cololejeunea contractiloba** sp. nov.

Plants very delicate, pale green, scattered or growing in loose mats: stems prostrate, 0.03 mm. in diameter, irregularly and sometimes abundantly branched, the branches widely spreading, similar to the stem: leaves distant to subimbricated, obliquely to widely spreading, the lobe plane or slightly convex, sometimes inflexed at the apex, ovate to ovate-lanceolate, when well developed 0.2–0.3 mm. long and 0.12–0.18 mm. wide, but often considerably smaller, gradually narrowed to an acute apex tipped with a single cell, both dorsal and ventral margins rounded in the basal half and straight or nearly so in the apical half, crenulate or denticulate from projecting cells; lobule often rudimentary, when well developed broadly ovate, about 0.13 mm. long and 0.11 mm. wide, strongly inflated throughout, apical tooth consisting of a single rounded projecting cell, lying in a more ventral plane than the rest of the free margin and bearing the hyaline papilla at its dorsal base, proximal tooth scarcely evident, consisting of a rounded cell separated from the apical tooth by a single cell, sinus

[1] Contribution from the Osborn Botanical Laboratory.

[2] Bryologist 18: 19–22. 1915.

shallow and very short; cells of lobe averaging 10 μ along the margin, 16 x 14 μ in the median and basal portions, each bearing a conical papilla on the dorsal surface, walls slightly thickened at the tips of the papillae, otherwise thin throughout; cells of lobule plane; stylus none: inflorescence autoicous: ♀ inflorescence borne on a somewhat elongated branch, innovating on one side, the innovation short and

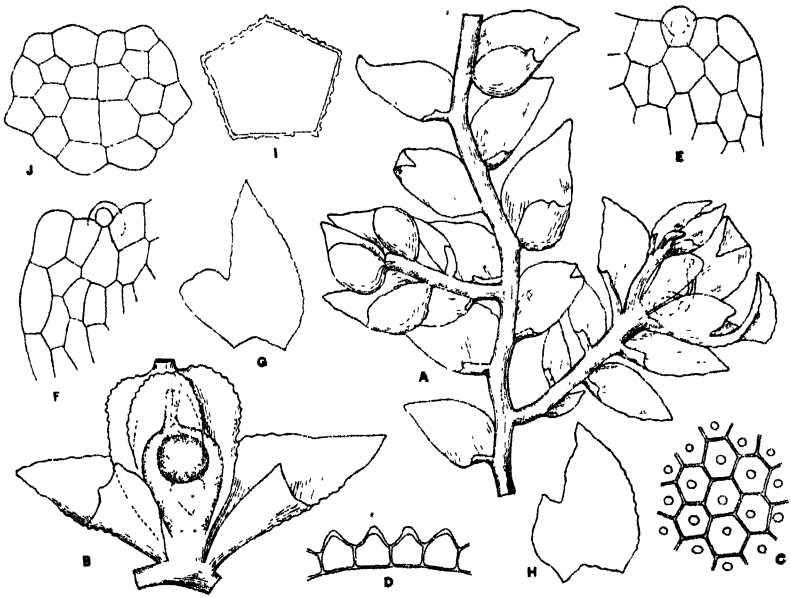


FIG. 1. COLOLEJEUNEA CONTRACTILOBA Evans

A. Part of a plant with male and female inflorescences, ventral view, × 65. *B*. Perianth and bracts, base of innovation at left, ventral view, × 65. *C*. Cells from middle of lobe, × 300. *D*. Cells of keel, optical section, × 300. *E*. Apex of lobule, ventral view, × 300. *F*. Apex of lobule, dorsal view, × 300. *G*, *H*. Bracts from a single involucre, × 65. *I*. Transverse section of perianth just above middle, × 65. *J*. Gemma, × 300. The figures were all drawn from the type specimen.

sterile; bracts obliquely spreading, more or less complicate, the lobe ovate to ovate-lanceolate, mostly 0.35–0.45 mm. long and 0.12–0.2 mm. wide, apex, margin and cells as in the leaves, lobule ovate to obovate, mostly 0.15–0.2 x 0.1 mm., rounded to more or less pointed at the apex; perianth obovate in outline, about 0.35 mm. long and 0.25 mm. wide, terete in lower half, five-keeled above, the keels blunt below, sharper above, rounded at the apex, beak of perianth short but distinct, surface in upper part roughened as in the leaves: ♂ inflorescence terminal on a more or less abbreviated branch, not pro-

liferating (so far as observed); bracts in one or two pairs, similar to the leaves but less widely spreading and with relatively shorter lobes, monandrous: capsule about 0.18 mm. in diameter: spores greenish, 12–20 μ in short diameter, minutely verruculose: gemmae abundantly produced, about 0.05 x 0.06 mm., composed (normally) of twenty cells, each apical quadrant cutting off three segments, margin crenulate from projecting cells, the youngest two segments on each side sharper, organs of attachment none. [FIG. 1.]

On bark of trees. FLORIDA: Sanford, 1913–1917, *S. Rapp*. The specimen collected in 1915 (September 28), which bears well-developed perianths, may be designated the type. The specimen collected in 1913 was at first referred by the writer to *C. Biddlecomiae* and is reported under this name by Miss Haynes.

Among the species of *Cololejeunea* known from Florida, *C. Biddlecomiae* (Aust.) Evans and *C. tuberculata* Evans agree with *C. contractiloba* in having roughened leaves and perianths. In all three cases the roughness is due to projecting cell-walls, more or less thickened at the tips of the projections. It is best marked in *C. tuberculata*, where the lobules as well as the lobes of the leaves and perichaetial bracts are usually roughened and where the projections are longer and more thickened at their tips. In the other two species the lobules are invariably smooth, and the projections are shorter and less thickened.

The lobules of the new species are especially interesting because they show the features characteristic of the genus in an abridged or reduced form. In other words the apical tooth, instead of being two cells or more long, consists of a single projecting cell, while the proximal tooth is scarcely apparent. The apical tooth is further remarkable because it lies in a more ventral plane than the rest of the free margin, the hyaline papilla lying in the same plane. In both *C. Biddlecomiae* and *C. tuberculata* the apical tooth is normally two cells long and lies in the same plane as the rest of the margin, while the proximal tooth is usually distinct.

Aside from the differences just noted *C. contractiloba* differs from *C. Biddlecomiae* in its smaller size, in its lack of a filiform stylus, and in the narrower lobes and lobules of its perichaetial bracts; it differs from *C. tuberculata* in its slightly larger size, in the entire lobules of its perichaetial bracts and in the distinct beak of its perianth. Four other speceis of *Cololejeunea* are definitely known from Florida at the present time. Since, however, the leaves of all are smooth or nearly so, there is little danger of confusing them with the present species.

2. **Lejeunea cladogyna** sp. nov.

Pale or dull green, sometimes becoming yellowish or brownish with age, growing in loose mats: stems mostly 0.08–0.1 mm. in diameter, sparingly and irregularly pinnate, the branches widely spreading and sometimes subdivided, often with smaller leaves than the stem but not microphyllous: leaves contiguous to loosely imbricated, the lobe obliquely to widely spreading, plane or slightly convex, subfalcate, broadly ovate, when well developed about 0.45 mm. long and 0.4 mm. wide, dorsal margin sometimes arching partially across the axis, sometimes not, sometimes strongly outwardly curved from the base to the broad and rounded apex, sometimes straight or slightly incurved in the basal region, ventral margin slightly outwardly curved to straight, margin entire throughout; lobule when well developed inflated, broadly ovoid, 0.09–0.12 mm. long and 0.09 mm. wide, keel straight or slightly arched, roughened from projecting cells, forming a very broad angle with the ventral margin of lobe, free margin somewhat involute to beyond the apex, sinus oblique and very shallow, apical tooth a rounded, straight, slightly projecting cell with a hyaline papilla or its proximal side; lobule usually poorly developed and reduced to a minute basal fold; cells of lobe averaging about 16 μ along the margin and 28 x 18 μ in the median and basal portions, thin-walled throughout or with minute and indistinct trigones and intermediate thickenings, cuticle smooth; ocelli none: underleaves distant, ovate to ovate-orbicular, about 0.15 mm. long and 0.12–0.15 mm. wide, bifid about one half with an acute to lunulate sinus and erect, triangular, subacute lobes, margin entire: inflorescence autoicous: ♀ inflorescence borne on a very short branch, with only one vegetative leaf and one bracteole in addition to the involucral leaves, innovating on one side, the innovation short and sterile (so far as observed); bracts obliquely spreading, the lobe oblong to obovate, when well developed about 0.3 mm. long and 0.16 mm. wide, rounded at the apex, entire, lobule about 0.2 mm. long and 0.08 mm. wide, narrowly oblong, the free portion 0.06 mm. long or less, obtuse to rounded at the apex; bracteole somewhat connate on one or both sides, oblong, about 0.3 mm. long and 0.16 mm. wide, bifid about one fourth with a narrow sinus and erect, rounded or very obtuse lobes, margin otherwise entire; perianth long-exserted, narrowly obovoid, about 0.6 mm. long, and 0.3 mm. wide, truncate at the apex and with a short beak, narrowed toward the base, sharply five-keeled above the middle, the keels indistinctly crenulate or denticulate from projecting cells, surface otherwise smooth: ♂ inflorescence occupying a short branch, not proliferating; bracts in two or three pairs, closely imbricated, strongly inflated, shortly bifid with a strongly arched, crenulate keel, and blunt divisions; antheridia in pairs; bracteole usually single at the base of the inflorescence, minute, shortly bifid: mature capsule about 0.2 mm. in diameter. [FIG. 2.]

On trees, logs, and sandy banks. FLORIDA: Caloosa, without date, *C. F. Austin;* Sanford, 1906–1913, *S. Rapp 6, 19, 64, 64a, 64b, 69;* Gainsville, 1916, *N. L. T. Nelson 104.* PORTO RICO: near Santurce, 1899, *Mr. & Mrs. A. A. Heller 616, 1365;* near Mayaguez, 1906, *E. G. Britton & D. W. Marble,* mixed with *542;* near Mayaguez, 1914, *E. G. Britton 1906.* Mr. Rapp's No. 64 may be designated the type. The specimens collected by Austin are in the Underwood

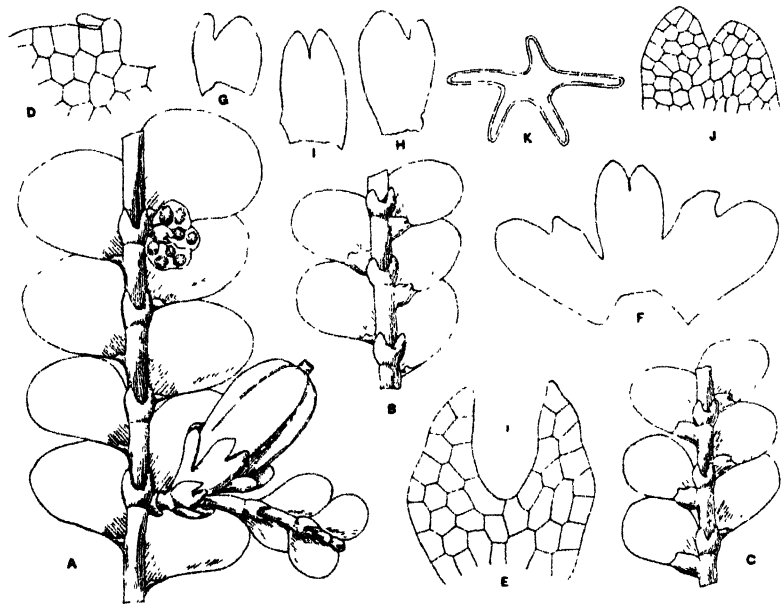


FIG. 2. LEJEUNEA CLADOGYNA Evans

A. Part of a robust plant with perianth and male inflorescence, ventral view, × 40. *B, C*. Parts of sterile plants, showing well-developed lobules, ventral view, × 40. *D*. Apex of lobule, × 225. *E*. Underleaf, × 225. *F*. Bracts and bracteole, × 50. *G–I*. Bracts and bracteole from another involucre, torn apart, × 50. *J*. Apex of bracteole shown in *F*, × 100. *K*. Transverse section of a perianth in upper third, × 50. The figures were all drawn from the type specimen.

Herbarium, now belonging to the New York Botanical Garden. In spite of their sterility Austin recognized their distinctness and gave them a manuscript name. Since this name has since been applied to a species from New Caledonia it is not available for the American plant.

In many species of *Lejeunea* the female branches vary greatly in length. This is strikingly true in the case of *L. minutiloba* Evans, a species of the West Indian lowlands, closely related to *L. cladogyna*. A female branch in this species is sometimes greatly elongated and sometimes so short that it bears a single vegetative leaf and a single underleaf in addition to the bracts and bracteole. Between these two extremes all intermediate conditions occur. In *L. cladogyna* the female branches, so far as observed, are always very short and conform to the second of the two extremes noted under *L. minutiloba*. It would perhaps be premature to state that this condition is absolutely constant, but it is certainly predominant, and it therefore seems justifiable to regard it as one of the distinctive characters of the species.

In size and in general habit *L. cladogyna* and *L. minutiloba* resemble each other very closely, and the inflorescence in both species is autoicous. In *L. cladogyna*, moreover, the lobule is usually reduced to a minute basal fold. While, however, this condition seems to be constant in *L. minutiloba*, inflated lobules of the usual *Lejeunea* type are occasionally produced in *L. cladogyna*, although many plants seem to lack them completely. The new species is further distinguished by its underleaves, bracts and perianths. The underleaves, even, when well developed, are only a little broader than the stem and the divisions are rarely more than four cells wide at the base; the lobules of the bracts are highly connate with the lobes and sometimes approximate them in length; the perianth is rounded at the apex, and the five keels extend to the middle or beyond. In *L. minutiloba* the underleaves are often twice as broad as the stem and the divisions may be six or more cells wide; the lobules of the bracts are less highly connate with the lobes and much shorter, appearing like small basal appendages; the perianth is truncate or slightly retuse at the apex, and the keels are restricted to the apical portion.

In *L. glaucescens* Gottsche, another West Indian species found also in Florida, the female branch seems to be constantly very short, just as in *L. cladogyna*, and the two species agree further in their autoicous inflorescence and in the fact that their lobules are often poorly developed. *L. glaucescens*, however, is a larger and more delicate species than *L. cladogyna* and has larger leaf-cells, the median cells of the lobes averaging about 33 μ in length. It is further distinguished by its sharper lobules and by the sharper divisions of its bracteoles.

Two other species of *Lejeunea*, *L. floridana* Evans and *L. flava* (Sw.)

Nees, are known from Florida. *L. floridana* agrees with *L. glaucescens* in most of its vegetative characters, but is distinguished from it by its much larger bracts and bracteole, by the short keels of its perianth (projecting slightly upward as horns), and by the fact that the female branches are often long. These features will serve to separate the species also from *L. cladogyna*. *L. flava* is distinguished by its larger size, by its much larger underleaves (which are often imbricated), by the variable length of its female branches, and by its usually well-developed lobules of the *Lejeunea* type.

3. LEJEUNEA LONGIFISSA Steph.

Lejeunea longifissa Steph. Sp. Hepat. 5: 747. 1915. [FIG. 3.]

On bark of trees. FLORIDA: Sanford, March, 1917, *S. Rapp 83*. CUBA: Monte Verde, February, 1859, *C. Wright*. The type material was collected in Cuba, no further data being given by Stephani. Since the type has not been available for comparison, the writer has been dependent upon the original description, which agrees in all essential respects with the specimens listed above.

The plants are pale green and cling closely to the substratum, forming thin irregular mats. As in so many of the Lejeuneae the best development of the leaves is found on sterile branches, rather than on those bearing sexual organs. In the latter position the lobules of the leaves are often imperfectly formed, although they rarely show the extreme reduction found in *L. cladogyna* and *L. minutiloba*. On sterile branches the leaves are loosely arranged and sometimes do not overlap at all. The lobes are plane or nearly so and spread obliquely. They are broadly ovate and slightly falcate, measuring, according to Stephani, 0.67 x 0.4 mm. The Florida specimens do not attain these dimensions, the largest lobes being about 0.4 x 0.3 mm., but the Cuban specimens have lobes 0.3–0.6 mm. in length. The apex of the lobe varies from rounded to very bluntly pointed, while the margin is entire or vaguely sinuate. The lobule, when well developed, is strongly inflated throughout, broadly ovate in outline, and measures about 0.12 x 0.1 mm. The free margin is involute as far as the apical tooth, which consists of a single, slightly projecting, blunt cell, with the usual hyaline papilla on the proximal side. The leaf-cells have thin walls with distinct trigones and frequent intermediate thickenings. According to Stephani the marginal cells measure 18 μ, the median 27 μ, and the basal cells 45 x 27 μ, these measurements agreeing with those made by the writer.

The underleaves are small and distant and show in general an orbicular outline. They are deeply bifid with an obtuse to lunulate sinus and erect or incurved lanceolate divisions, tipped with one or two cells and usually four cells wide at the base. The lateral margins are entire or vaguely and bluntly unidentate on the sides. The underleaf just below a perichaetial bracteole is usually larger than the others, with slightly broader divisions.

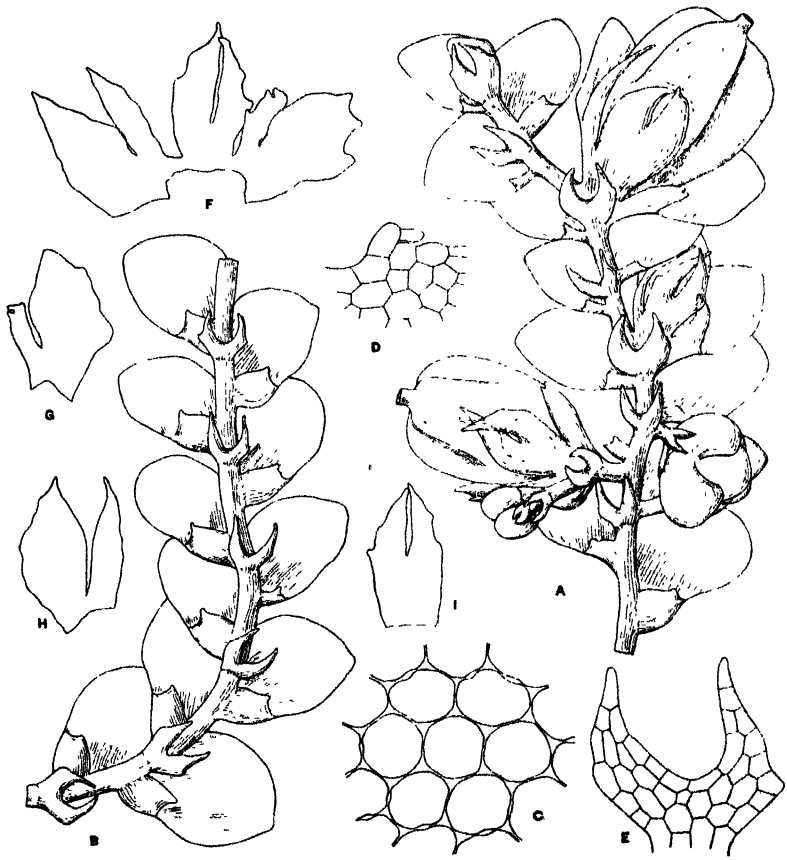


FIG. 3. LEJEUNEA LONGIFISSA Steph.

A. Part of plant with two perianths and a male inflorescence, ventral view, × 50. *B*. Part of a sterile stem, ventral view, × 50. *C*. Cells from middle of lobe, × 300. *D*. Apex of lobule, × 225. *E*. Underleaf, × 225. *F*. Bracts and bracteole, × 50. *G–I*. Bracts and bracteole from another involucre, torn apart, × 50. The figures were all drawn from Mr. Rapp's specimens, No. 83.

The inflorescence is autoicous, as in all the other species of *Lejeunea* known from the United States. The female inflorescence is sometimes borne on a short branch and sometimes on a more or less elongated branch. It innovates on one side and occasionally on both, the innovations being sometimes short and sterile, sometimes again florigerous. The bracts are exceedingly variable. The lobe of the outer bract is usually broad and blunt, the margin varying from entire to coarsely and irregularly sinuate or toothed. The lobe of the inner bract is usually narrow and sharper. The lobule of the outer bract is also blunt in most cases and often shows two indistinct teeth at the apex; the lobule of the inner bract is usually slender and long-pointed. Unfortunately these differences between the bracts are not always apparent. The bracteole is slightly connate on both sides; it is deeply bifid with a narrow sinus and slender, long-pointed divisions, and the margin is sometimes sparingly and irregularly toothed. The perianth is obovoid and distinctly five-keeled, the dorsal keel being shorter than the two ventral. The keels are crenulate and sometimes show very narrow and interrupted wings. The apex of the perianth is rounded or truncate and the beak is distinct. The measurements of the involucral leaves and perianths which Stephani gives are somewhat higher than those made by the writer. According to him the lobes of the bracts measure 0.9 x 0.45 mm. while the perianth is said to be 1.25 mm. long and 0.67 mm. wide. In the writer's experience the lobes of the bracts measure 0.35–0.7 x 0.22–0.35 mm., and the perianth 0.5–0.9 x 0.35–0.5 mm. Stephani speaks of the perianth as being "quasi pedunculata," so that his measurements were evidently made from plants which had passed maturity. In view of the great variation in size exhibited by the bracts and perianths, the discrepancies just noted hardly seem sufficient to warrant a specific separation. The male spikes vary in position and in length and apparently never proliferate. They sometimes occupy short branches and sometimes terminate long branches, and the bracts are mostly in two to six pairs. The antheridia are borne singly or in pairs.

Perhaps the most striking features of *L. longifissa* are the deeply bifid underleaves, from which it receives its specific name, and the variable perichaetial bracts, some of which at least have sharp-pointed and coarsely toothed lobes. In all the other species of *Lejeunea* known from Florida the lobes of the bracts are either rounded or very bluntly pointed, while their margins are entire or vaguely

crenulate. The underleaves with their lanceolate divisions resemble somewhat those of *L. pililoba* Spruce and *L. spiniloba* Lindenb. & Gottsche, but they agree even better with those of *Microlejeunea laetevirens* (Mont. & Nees) Evans on account of the fact that they are sometimes unidentate on the sides.

4. Rectolejeunea Maxonii Evans

Rectolejeunea Maxonii Evans, Bull. Torrey Club **39**: 609. *pl. 45, f. 17–27.* 1912.

On bark of trees. Florida: Gainesville, March, 1916, *N. L. T. Nelson 79, 92;* Robinson's Spring, eight miles south of Sanford, April, 1917, *S. Rapp 87.* Alabama: Auburn, September, 1900, *F. E. Lloyd & F. S. Earle.* Porto Rico: Mount Morales, near Utuado, March, 1906, *M. A. Howe 453.* The species was based on the following specimens, collected in 1903, at Cinchona, Jamaica: *L. M. Underwood 495; W. R. Maxon 1361* (type); *A. W. Evans 143* in part. No other stations are at present known.

The specimens from Florida and Alabama bear numerous female inflorescence but show neither perianths nor androecia. Since they are slightly smaller than the original material of *R. Maxonii* from Jamaica, it at first seemed unwarranted to refer them definitely to that species. The specimens from Porto Rico, however, bridge over the gap. Most of them are no larger than the plants from the United States, while others equal the Jamaican plants in size. In other respects the specimens from the different localities show an essential agreement. In all probability the range of *R. Maxonii* will be still further extended, now that its characteristics are more accurately understood.

In the original account of the species vegetative reproduction by means of caducous leaves was described but was reported as a rare phenomenon. The Florida specimens show that this is by no means the case. The majority of the stems examined have produced caducous leaves in abundance, and some of them have become almost leafless. In most instances plants of this character are sterile, but an occasional archegonium is produced, the bracts remaining firmly attached. When archegonia are abundant, the tendency to form caducous leaves is much less evident and seems to become completely inactive in plants with perianths. When the species was first proposed as new the lobes of the vegetative leaves were said to measure

0.5 x 0.4 mm. This size is rarely attained in the Porto Rican specimens, and then only in the vicinity of perianths. Most of the leaves are only 0.3–0.35 mm. in length by 0.3 mm. or less in width. Similar but less constant discrepancies are to be found in the perianths. One interesting peculiarity of the perichaetial bracteoles should be alluded to. Although their lateral margins may be entire or nearly so, as the original description perhaps implies, it is more usual for them to be irregularly toothed, and in many cases a single large tooth on each side can be demonstrated.

The close relationship between *R. Maxonii*, *R. phyllobola* (Nees & Mont.) Evans and *R. Brittoniae* Evans was emphasized in the original publication of the species, and the differential characters were there contrasted. The dioicous inflorescence will at once distinguish *R. Maxonii* from the autoicous *R. phyllobola*. From *R. Brittoniae*, which is likewise dioicous, the best differential characters are drawn from the androecia. In *R. Brittoniae* these bear bracteoles along their entire length; in *R. Maxonii* at the base only. In the absence of androecia, the smaller size and paler color of *R. Maxonii* will usually serve to distinguish it.

5. **Euosmolejeunea parvula** sp. nov.

Pale or dull green, sometimes becoming brownish with age, scattered or growing in loose mats; stems mostly 0.06–0.08 mm. in diameter, copiously and irregularly branched, the branches widely spreading and often subdivided, not microphyllous: leaves imbricated, the lobe obliquely to widely spreading, plane or slightly convex, falcate, broadly ovate, mostly 0.25–0.35 mm. long and 0.2–0.3 mm. wide, dorsal margin arching partially or wholly across the axis, sometimes straight at base but usually strongly outwardly curved from the base to the broad and rounded apex, ventral margin straight or slightly outwardly curved, forming a straight line or a very obtuse angle with the keel, margin entire throughout; lobule inflated throughout, ovoid, about 0.1 mm. long and 0.07 mm. wide, keel straight to somewhat arched, roughened from projecting cells, free margin strongly involute to beyond the apex, sinus oblique and shallow, apical tooth a single, blunt, projecting cell with a hyaline papilla in a slight depression at its distal base; cells of lobe about 12 μ in diameter at the margin and 20 x 16 μ in the median and basal portions, thin-walled but usually with minute trigones, cuticle smooth; ocelli none: underleaves distant to contiguous, broadly ovate to orbicular, when well developed mostly 0.12–0.16 mm. long and 0.12–0.14 mm. wide, bifid about one half with a sharp and often narrow sinus and erect triangular lobes, obtuse to subacute at the apex,

margin entire: inflorescence autoicous: ♀ inflorescence borne on a more or less abbreviated branch, sometimes with only a single vegetative leaf and a single underleaf in addition to the bracts and bracteole, innovating on one side, the innovation short and usually sterile but sometimes bearing a second ♀ inflorescence; bracts obliquely spreading, sharply or bluntly keeled, the lobe falcate, ovate to obovate, about 0.45 mm. long and 0.3 mm. wide, rounded to very bluntly pointed at the apex, margin entire or vaguely sinuate, narrowly oblong, about 0.3 mm. long and 0.09 mm. wide, the free portion scarcely 0.06 mm. long, rounded to acute; bracteole free, ovate-elliptical, about 0.4 mm. long and 0.3 mm. wide, bifid about one third with a narrow sinus and erect or connivent lobes, obtusely to acutely pointed, margin entire or vaguely crenulate; perianth about half exserted, obovoid, mostly 0.6–0.7 mm. long and 0.45 mm. wide, cuneate toward the base, rounded to truncate at the apex with a short beak, five-keeled, dorsal keel shorter and blunter than the others, extending scarcely to the middle, lateral keels sharp, ventral keels usually united into a broad two-angled keel, lateral and ventral keels sometimes very narrowly and vaguely winged, slightly roughened from projecting cells, surface of perianth otherwise smooth: ♂ inflorescence terminal on a more or less elongated branch or occupying a short branch, sometimes proliferating; bracts mostly in four to six pairs, imbricated, about as large as the vegetative leaves, strongly inflated, shortly bifid with a rounded dorsal lobe, a pointed ventral lobe, and a strongly arched keel slightly roughened from projecting cells; antheridia in pairs; bracteoles mostly two at the base of the inflorescence, similar to the underleaves: mature capsule about 0.2 mm. in diameter. [FIG. 4.]

On bark. FLORIDA: Sanford, January, 1917, *S. Rapp 86;* Robinson's Spring, eight miles south of Sanford, May, 1917, *S. Rapp 86a* No. 86 may be designated the type.

In discussing the genus *Cheilolejeunea* several years ago the writer[3] called attention to the fact that its relationship to *Euosmolejeunea* was uncomfortably close. Typical species of *Cheilolejeunea*, to be sure, are clearly distinct from typical species of *Euosmolejeunea*, but other species occupy an intermediate position and might be placed in the one genus almost as well as in the other. The present species is a case in point. In its small size, general habit, foliar characters and small underleaves it agrees with *Cheilolejeunea* better than with *Euosmolejeunea*, but its five-keeled perianth indicates that it should be referred to the latter genus. Possibly, when the species of the two genera are more thoroughly understood, it may be advisable to include them under a single genus.

[3] Bull. Torrey Club 33: 5. 1906.

The closest known relative of *E. parvula* is *E. duriuscula* (Nees) Evans, another species on the border line between *Cheilolejeunea* and *Euosmolejeunea*. *E. duriuscula* is widely distributed in tropical and subtropical America and occurs abundantly in Florida. It is only a

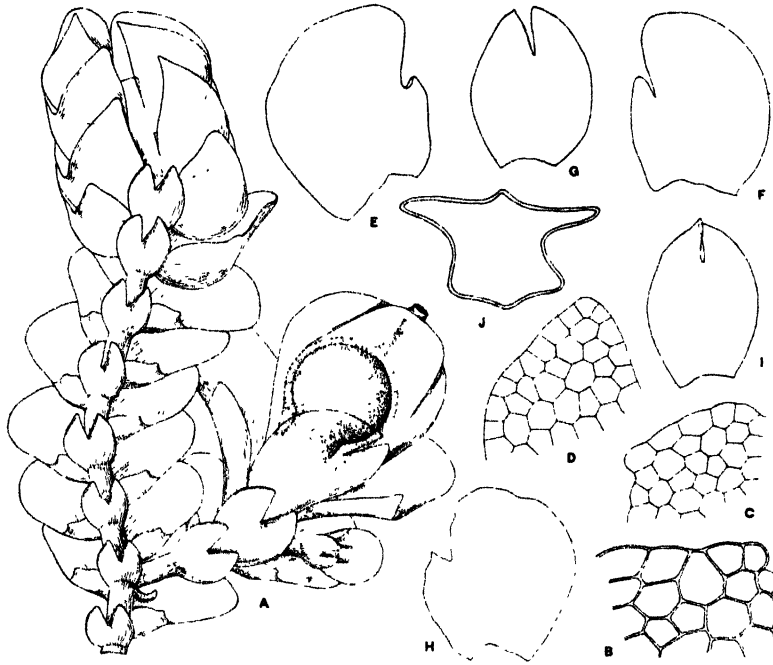


FIG. 4. EUOSMOLEJEUNEA PARVULA Evans

A. Part of a plant with perianth and male inflorescence, ventral view, × 50. *B*. Apex of lobule, showing distal hyaline papilla, × 300. *C*. Apex of another lobule, papilla not shown, × 225. *D*. Apex of an underleaf-division, × 225. *E–G*. Bracts and bracteole from a single involucre, × 50. *H*, *I*. Bract and bracteole from another involucre, × 50. *J*. Transverse section of a perianth in upper third, × 50. The figures were all drawn from the type specimen.

trifle larger than *E. parvula*, the lobes of its leaves measuring about 0.4 x 0.35 mm., the lobules in the two species are much like the apical tooth, being very short in both, the underleaves and leaves are very similar, and the perianths, except for a slight difference in size, agree closely. *E. duriuscula*, however, is a yellowish plant of a firmer texture, the leaf-cells have larger and more distinct trigones, the female

inflorescence is usually borne on a leading branch, and the lobules of the perichaetial bracts are relatively broader and separated from the lobes by deeper sinuses. An even more important difference than any of these is found in the autoicous inflorescence of the new species, *E. duriuscula* being invariably dioicous.

One other species of *Euosmolejeunea*, the widely distributed *E. clausa* (Nees & Mont.) Evans of tropical and subtropical America, is likewise known from Florida. This species agrees with *E. duriuscula* in its dioicous inflorescence but is characterized by its larger underleaves, distinctly rounded or cordate at the base, and by the fact that the female inflorescences are borne on more or less abbreviated branches. The dioicous inflorescence and the underleaves would at once separate *E. clausa* from *E. parvula*, although the short female branches might suggest a relationship. It is further distinguished by its yellowish-green color, by its larger size (the leaf-lobes measuring about 0.5 x 0.4 mm.), and by its firmer texture, the leaf-cells being provided with distinct trigones, just as in *E. duriuscula*.

Other Florida species with which *E. parvula* might perhaps be confused are *Cheilolejeunea polyantha* Evans and *Rectolejeunea phyllobola* (Nees & Mont.) Evans. In the *Cheilolejeunea* the inflorescence is dioicous, the leaves are densely imbricated, the lobes are orbicular and measure about 0.4 mm. in diameter, the underleaves are often broader than long and are rounded or cordate at the base, and the dorsal surface of the perianth is practically without a keel. All of these features would separate it from the new species. The *Rectolejeunea* agrees with *E. parvula* in its autoicous inflorescence but is a somewhat larger plant when well developed and is further distinguished by the proximal position of the hyaline papilla associated with the apical tooth of the lobule, by the lack of a dorsal keel on the perianth and by slight differences in the form of the underleaves, bracts and bracteoles.

6. **Ptychocoleus heterophyllus** sp. nov.

Yellowish or brownish green, scattered or growing in depressed mats: stems 1.5–2 mm. in diameter, sparingly pinnate, the branches obliquely to widely spreading, usually of the *Radula* type, rarely of the *Frullania* type, similar to the stem: leaves loosely to closely imbricated, squarrose when moist, the lobe falcate, ovate, 0.6–0.75 mm. long, 0.45–0.6 mm. wide, rounded at the dorsal base, then strongly outwardly curved to the rounded or very obtuse apex, margin entire; lobule broadly ovate-triangular when explanate, 0.35 mm. long,

0.3 mm. wide, the inflated portion forming a narrowly ovate water-sac, keel strongly arched near the base, then almost straight and forming a very wide angle with the slightly involute ventral margin of the lobe, free margin rounded at the base, then almost straight to junction with lobe (including the apical sinus), bearing usually from five to seven short and strongly inflexed blunt teeth, each consisting of a single projecting cell and separated from its neighbors by about two cells, apical tooth like the others, hyaline papilla proximal to the apical tooth and situated on the dorsal surface of the second cell from the margin, cells of lobe more or less convex, averaging about 13 μ at the margin, 25 x 20 μ in the middle, and 32 x 16 μ at the base, trigones distinct, triangular, mostly with two convex sides and one concave side, intermediate thickenings infrequent, oval: underleaves loosely to closely imbricated, plane, broadly orbicular, mostly 0.3–0.35 mm. long and 0.35–0.4 mm. wide, apex rounded to truncate, base shortly cuneate, rounded, or minutely and indistinctly auriculate, margin entire: inflorescence dioicous: ♂ inflorescence at first terminal, afterwards proliferating; bracts mostly in six to ten pairs, closely imbricated, similar to the leaves but the lobe relatively broader, lobule with a broader inflated portion, ovate, truncate at the outer end, the sinus forming about a right angle with the rest of the free margin, apical tooth one or two cells long, not inflexed, margin otherwise entire or nearly so; bracteoles similar to the underleaves; antheridia in pairs: vegetative reproduction by means of small caducous leaves borne on specialized upright branches with persistent squarrose underleaves: ♀ plant unknown. [FIG. 5.]

On bark of trees. FLORIDA: Sanford, March, 1911, and May, 1912, *S. Rapp;* Robinson's Spring, eight miles south of Sanford, May, 1917, *S. Rapp.* HONDURAS: in deep swamp along Highland Creek, near Puerto Sierra (Tela), at about sea-level, February, 1903, *P. Wilson 569.* The Florida plants lack both antheridia and archegonia; the Honduras specimens bear antheridia only. Mr. Rapp's specimen, collected in 1917, may be designated the type.

The close relationship existing between *Ptychocoleus* and *Brachiolejeunea* has already been emphasized by the writer in another connection.[4] In their vegetative organs the two genera are essentially alike, and the only constant difference between them is the absence of subfloral innovations in *Ptychocoleus* and their presence in *Brachiolejeunea.* Since the plants just described are wholly without archegonia it is clearly impossible to determine their generic position beyond all question. They are referred to *Ptychocoleus* largely on account of their caducous leaves, borne on specialized branches, the leaves

[4] Bull. Torrey Club 35: 161, 162. 1908.

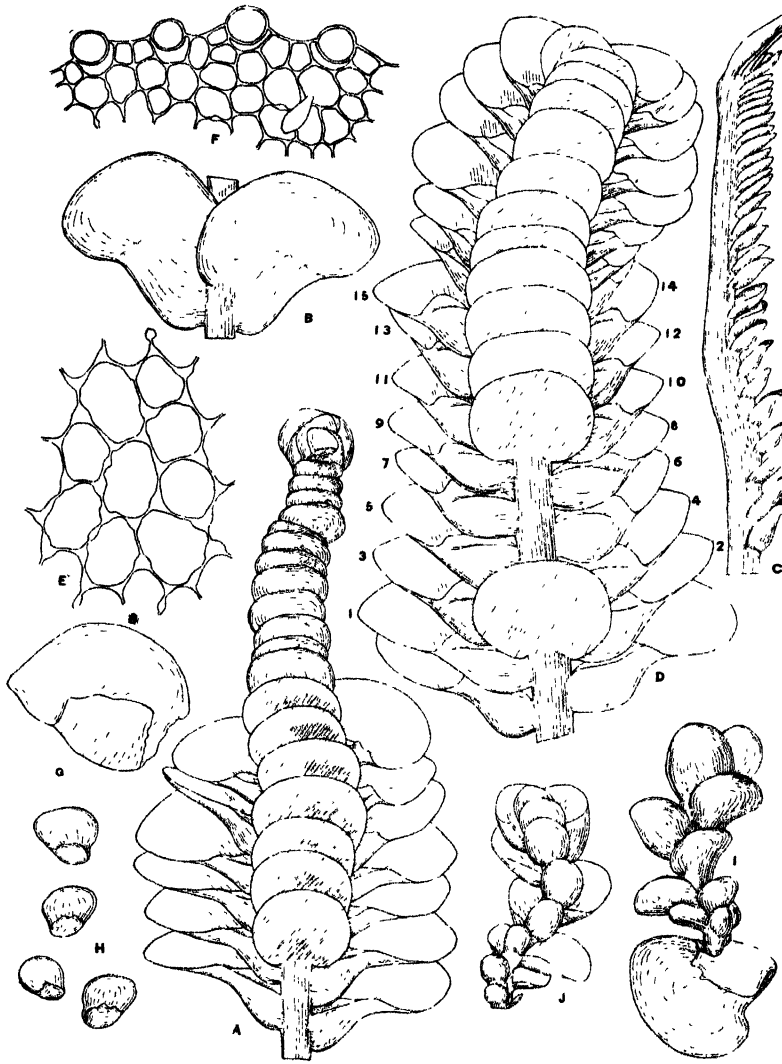


FIG. 5. PTYCHOCOLEUS HETEROPHYLLUS Evans

A. Part of a branch, the apical portion specialized, × 40. *B*. Two vegetative leaves, dorsal view, × 40. *C*. Specialized branch, lateral view, × 40. *D*. Part of plant, including a male inflorescence, the male bracts numbered 1–15, ventral view, × 40. *E*. Cells from middle of lobe, × 300. *F*. Part of lobular margin, the apical tooth at right, × 225. *G*. Male bract, × 40. *H*. Caducous leaves, × 40. *I*. Caducous leaf bearing a new shoot, × 50. *J*. Ventral view of the same shoot, × 50. *A*, *B*, *E* and *F* were drawn from the type specimen; *C*, *H*, *I* and *J*, from the specimen collected by Mr. Rapp in 1912; *D* and *G*, from the Honduras specimen.

serving as organs of vegetative reproduction. Such leaves and branches are unknown in *Brachiolejeunea* but are found in the South American *P. torulosus* (Lehm. & Lindenb.) Trevis.,[5] as understood by Spruce, although their true significance has been overlooked.

Vegetative reproduction by means of leaves which become separated and which afterwards give rise to new shoots by a process of regeneration are now known in several genera of the Hepaticae. In most cases the deciduous leaves are essentially like ordinary leaves and the line of separation is irregular. Such leaves are "Bruchblätter," according to the definition of Correns.[6] In rarer cases the leaves are distinctly modified and separate by means of a regular and definite line. Such leaves are "Brutblätter." Examples of the latter have been described by the writer in *Rectolejeunea flagelliformis* Evans and *R. Berteroana* (Gottsche) Evans,[7] and their occurrence has been noted in *Frullania Bolanderi* Aust.[8]

The caducous leaves of *Ptychocoleus heterophyllus* are likewise Brutblätter. Although they show the usual differentiation into lobe and lobule, both are greatly reduced in size, the lobe measuring about 0.25 x 0.2 mm. and the lobule 0.14 x 0.09 mm. The latter is further distinguished by bearing only one or two marginal teeth, not inflexed as on ordinary leaves. The separation takes place at the very base and no cells are torn across in the process. After separation the basal cells project as minute crenulations.

The branches which bear the caducous leaves vary greatly in length but their growth is limited sooner or later, and no evidence is at hand that they ever revert to the typical vegetative condition. In one case thirty pairs of leaves had been produced. The transition between ordinary leaves and caducous leaves is abrupt; as soon as the latter begin to be formed the branch curves away from the substratum and ceases to form rhizoids. The persistent underleaves are much like ordinary underleaves and their reduction in size is less marked than in the case of the leaves. They are very densely crowded, however, and are distinguished also by being squarrose and more or

[5] In his Species Hepaticarum (5: 37. 1912) Stephani cites the present writer as authority for this combination with the reference, "Torr. Bot. Cl., 1908, p 165." If this reference is consulted it will be seen that the combination is correctly assigned to Trevisan.

[6] Unters. über die Vermehrung der Laubm. 338. 1899.

[7] Bull. Torrey Club 33: 10, 13. 1906.

[8] Bryologist 18: 88. 1915.

less convex. At the apex of the branch the few leaves which are still attached bend backward almost as strongly as the underleaves. After the leaves have fallen away the surface of the branch appears irregularly roughened from projecting cells, but it is difficult to determine the actual lines of attachment. The upright leafless branches, with their persistent and crowded underleaves, present a very distinctive appearance. Aside from *P. torulosus* branches of this character have not been noted in the Lejeuneae Holostipae. They may be compared with the flagelliform branches found in *Frullania Bolanderi* and in the two species of *Rectolejeunea* noted above.

The behavior of the caducous leaves after they have fallen away was observed in but a single instance. In this case a new shoot had grown out from the lower surface of the lobule not far from the apical tooth. This shoot was leafy from the very base and had immediately formed undivided underleaves as well as leaves. The latter, although small, showed distinct lobules. In the few Lejeuneae where germination has been observed the spore first gives rise to a row of cells (sometimes very short), then to a flat thallus and finally to a leafy shoot. In certain other genera the leafy shoot at its beginning is destitute of underleaves and shows undivided leaves, even though the adult shoot bears well-developed underleaves and bilobed leaves. It is of interest to note that the shoot growing out of the caducous leaf in *P. heterophyllus* showed none of these embryonic features. At the same time it would be premature to draw any general conclusions from a single example, and it is probable that cases of more pronounced reversion may yet be discovered. It is also probable that the new shoots do not always arise from the lobule. In *Rectolejeunea flagelliformis*, where the caducous leaves lack lobules, the new shoots grow out from the margin of the lobes, and it would be natural to suppose that the *Ptychocoleus* might show the same phenomenon.

The lobular teeth in *P. heterophyllus* are usually five to seven and are remarkable for their uniform structure and regular spacing. On account of their being so strongly inflexed it is easy to overlook them, and their features can only be made out satisfactorily by careful dissection. Each tooth consists of a single projecting cell borne on a broader basal cell, and the apical tooth is indistinguishable from the others except by its position. The proximal tooth, however, is often less definite. The apical sinus, in explanate lobules, continues the

line of the free margin and does not form a distinct depression. On the underleaves basal auricles are occasionally present, but they are never well developed, and are always difficult to demonstrate.

The South American *P. torulosus* is still incompletely known and it is possible that the species, as at present defined, represents an aggregate. The type specimen was collected in "Guiana," and the species has since been reported from Dutch Guiana, from Venezuela, and from Brazil. In the Hepaticae Spruceanae specimens were distributed from Obidos, Brazil, and from the vicinity of Chimborazo, Ecuador. These and a portion of the type material in the Mitten herbarium have been available for study.

In the type specimen perianths are present but neither androecia nor caducous leaves were detected. The plants are considerably larger than those of *P. heterophyllus*, and the lobes of the leaves are relatively broader, measuring 1.1–1.3 mm. in length and 0.95–1.2 mm. in width. The ventral margin of the lobe is further distinguished by being distinctly revolute. The margin of the lobule is said to be entire in the original description, but the marginal teeth were soon noted by Lindenberg and Gottsche.[9] They usually number four to six and are less strongly inflexed than those of the new species but resemble them in other respects. The underleaves measure about 0.5 mm. in length and 0.75 mm. in width; in most cases they show small basal auricles, but these are not always distinct and may be absent altogether. A leaf, an underleaf, an involucre, and a perianth in cross section have been figured by Schiffner,[10] presumably from material in the Lindenberg Herbarium at Vienna.

Spruce's specimens are scarcely larger than those of *P. heterophyllus*, but their leaves agree in shape with those of the type from Guiana, measuring about 0.75 mm. in width and scarcely more than that in length. The underleaves, too, are much broader than long and usually show distinct auricles. The free margin of the lobule, however, offers a few distinctive features, when compared with the type. Although the number of teeth is about the same, the apical tooth is longer than the others and extends outward, instead of being inflexed, a distinct sinus being thus formed between the apical tooth and the distal portion of the margin. The other teeth are inflexed, but not very strongly so. The branches with caducous leaves are

[9] Linnaea **24**: 627. 1851.

[10] Hedwigia **33**: *pl. 7, f. 8–10.* 1894.

probably referred to by Spruce when he speaks of "rami decurvi apice subaphyllo." They agree in all essential respects with those of *P. heterophyllus*.

Two species of *Brachiolejeunea* are known from Florida at the present time, *B. corticalis* (Lehm. & Lindenb.) Schiffn.[11] and *B. bahamensis* Evans.[12] Both usually bear perianths in abundance with the subfloral innovations characteristic of the genus. They are both somewhat darker than the new *Ptychocoleus*, and are slightly more robust, their leaf-lobes measuring about 0.9 mm. in length. Further differences in the marginal teeth of the lobules may be noted. There are usually four of these teeth in *B. corticalis* and five in *B. bahamensis*, the teeth being only slightly inflexed, so that it is possible to flatten them out. In *B. corticalis* the teeth are relatively simple, but in *B. bahamensis* they are usually three or four cells in length and show considerable irregularity.

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[11] See Evans. Mem. Torrey Club **8**: 131. *pl. 18, f. 1–11.* 1902.
[12] Evans. Bull. Torrey Club **35**: 383. *pl. 28, f. 1–4.* 1908.

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COPPER AND ZINC AS ANTAGONISTIC AGENTS TO THE "ALKALI" SALTS IN SOILS

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Since the appearance of Osterhout's pointed reply[1] to Loew's criticism of the conception of antagonistic salt effects and physiologically balanced solutions for plants, no one has seriously questioned the validity of accepting as well-founded the aforementioned conception. Indeed the antagonistic salt effect is now regarded as one of the established facts in plant physiology, as it has been in animal physiology since 1900, when Loeb[2] first suggested the idea of the ion-proteid compounds and the mechanism of toxic and antagonistic salt effects. In the large amount of work which has been accomplished on the antagonistic effects of salts during the last fifteen years, the heavy metals have received very little attention, while the alkali and alkali-earth metals have been tested out in a number of ways and with a variety of media.

In the experiments on animals, which we have cited above, it was found by Loeb[3] that for the development of the eggs of marine fish (Fundulus) in NaCl solution of the same osmotic pressure as sea water, copper and mercury were powerless to antagonize the toxic effects of common salt. Zinc and cobalt, on the other hand, manifested a marked antagonism to NaCl in the direction indicated, and lead, nickel, and uranium showed slight but definite antagonistic powers under similar circumstances Experiments involving antagonistic

[1] Osterhout, W. J. V. The Nature of Balanced Solutions. Bot. Gaz. 47: 48. 1909.

[2] Loeb, J. On Ion-Proteid Compounds and their Rôle in the Mechanics of Life Phenomena. The Poisonous Character of a Pure NaCl Solution. Amer. Journ. Physiol. 3: 327. 1900.

[3] Loeb and Gies, Pflüger's Arch. 93: 246. 1902.

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powers of copper and zinc ions to the toxic properties of other ions have been few and the results obtained rather fragmentary. They have dealt chiefly with animal material. Indirectly, however, a small amount of data has been obtained in experiments with plant or fungus organisms as regards antagonistic powers of copper and zinc. We use the term *indirectly* advisedly, since, unlike our experiments, those in question have attempted to antagonize the toxic properties of copper and zinc by adding the less toxic or non-toxic metals to a given medium for the growth of the organism tested, whereas we have attempted to use copper and zinc to antagonize the toxic concentrations of what are known as "alkali" salts in soils. The indirect evidence is very important, however, and deserves mention here. Clark[4] was able to diminish markedly the toxic effects of $CuSO_4$ and $CuCl_2$ for germination of spores of *Oedocephalum albidum* and *Rhizopus nigricans* by the addition of various ammonium, sodium, and potassium salts. Among other heavy metals, Le Renard[5] found that copper and zinc could be rendered much less toxic in culture media for Penicillium by the addition of various salts of ammonium, potassium and magnesium. True and Gies[6] demonstrated that the toxicity of copper and zinc, as well as that of mercury in various salts for *Lupinus albus*, could be considerably reduced by the addition of calcium to the medium of growth. Szücs,[7] working with *Cucurbita pepo* and using the responsiveness of the root to a geotropic stimulus as a criterion, found that $AlCl_3$ in certain concentrations possessed the property of inhibiting the toxic effects of $CuSO_4$. More recently, Hawkins[8] has shown to exist certain cases of undoubted inhibition of the toxic effects, on fungus spores, of heavy metals, including copper and zinc, by the presence of calcium, magnesium, or potassium nitrates. It will be noted that only two experiments with higher plants are cited among the investigations just reviewed. Moreover, Szücs used a very unusual and less convincing criterion for antagonism effects, and True and Gies used calcium to antagonize copper, but did not try the antagonistic properties of copper against the alkali or alkali earth metals or their ions.

On the other hand, Lillie[9] found that copper, as well as several

[4] Clark, J. F. Bot. Gaz. 33: 26–48. 1902.
[5] Le Renard, Alf. Ann. Sci. Nat. Bot. IX. 16: 276–336. 1912.
[6] True & Gies, Bull. Torrey Club 30: 390–402. 1903.
[7] Szücs, Jos. Jahrb. Wiss. Bot. 52: 85–143. 1912.
[8] Hawkins, L. A. Physiol. Res. 1: 57–92. 1913.
[9] Lillie, R S. Amer. Journ. Physiol. 10: 419. 1904.

other toxic metals, possesses definite powers of antagonizing the toxic effects of NaCl on the normal existence and activation of cilia in the larvae of a marine annelid (Arenicola). With the exception of uranium, however, copper was the most feeble antagonistic agent to the action of NaCl just mentioned of thirteen metals tested.

In view of the negative results obtained with copper by Loeb, and the entire lack of data, in so far as the more important functions of plants are concerned, on the antagonistic action of that metal to the alkalies, we deemed it wise, among the different series of antagonism experiments carried out in our laboratory, to test the action of copper as an antagonistic agent to "alkali" salts in soils. This seemed particularly important in view of certain marked stimulating effects obtained by us[10] through the presence in the soil of copper, zinc and other metals, in the growth of barley in soil cultures. Owing to many similarities between the stimulating effects of copper and zinc in the studies just referred to, we decided to study the antagonistic powers of zinc, as well as those of copper, in the new experiments. The latter have now been completed and the results have, in many ways, been so striking as to justify their publication at this time. Our data constitute the first evidence, so far as we are aware, of the antagonistic action of copper and zinc to the toxic effects of "alkali" salts as regards the living cells of higher plants.[11]

Methods Employed in the Experiment

The plants used as indicators of the salt effects here studied were a selected strain of the Beldi variety of barley (*Hordeum vulgare*). They were grown in soil in 8-inch earthenware pots which were paraffined prior to the introduction of the soil. Twenty seeds were planted in every pot and the plants were later thinned to six plants per pot. As nearly as possible, optimum and uniform moisture conditions were maintained in all the soils. Some of the common salts of alkali soils, viz, NaCl, Na_2SO_4, and Na_2CO_4, were employed as toxic agents and were added on a percentage basis of the dry weight of the soils. The antagonistic agents were $CuSO_4$, $ZnSO_4$, $CuCl_2$, $ZnCl_2$ and $CuCO_3$, and were added to the salt-treated soils on the basis of parts per million of

[10] Lipman and Gericke, Univ. Cal. Publ. Agr. Sci. 1: 495–587.

[11] Hibbard, R. P., has shown that $CuSO_4$ and chloral hydrate antagonize each other, but such an instance of antagonism is not comparable with those which we furnish in this paper since chloral hydrate is an organic compound.

the dry weight of the soil. The toxic salt in every case was used in uniform concentration throughout a given series, whereas the antagonistic salt was used in varying concentrations. Except as otherwise stated below, all salts were mixed with the soils a few days prior to planting the seeds.

Two types of soil were employed, the Oakley blow sand and the Berkeley clay adobe. Both of these soils have frequently been described in papers issued from our laboratory.[12] It should be added that different lots of one and the same type of soil were used and these varied in producing power without treatment due to field conditions which need no discussion here. In any one series, however, soils from different lots were never mixed. Seven series of cultures in duplicate were grown on each soil type. The plants were grown to maturity, harvested, dried at 100° C., and weighed. The dry weights of both tops and roots were determined in every case, and in the case of the tops, separate determinations were also made of the dry weight of the straw and the grain. The results are given in the tables. For the sake of clearness, it is deemed best to consider briefly each series by itself.

Series I

$CuSO_4$ versus Na_2SO_4—Adobe Soil

Na_2SO_4 .5 percent constant—$CuSO_4$ varying

Three consecutive crops were grown in this series, the second crop being planted shortly after the first was harvested. The salt applications were, of course, made only once, namely, prior to the planting of the first crop. The results obtained with regard to yields of straw, grain, and roots are given in Tables I, II and III.

Straw Production.—Straw yields are evidently not very markedly influenced by the antagonistic effects of $CuSO_4$ to Na_2SO_4. This seems to be true especially in the first two crops. In the third crop, the effect is a little more marked in the direction indicated. On the other hand, the lack of agreement, which is noted between some of the duplicate cultures, is more marked in the third crop than in the other two. It should be observed that the lack of toxicity manifested by Na_2SO_4 alone in the second crop is doubtless due to a loss of some of the salt, since Na_2SO_4 is characterized by a tendency to crystallize from the soil and to creep to and over the edges of the pots. Moreover,

[12] Univ. Cal. Publ. Agr. Sci. 1: 495–587.

any spots on the pot from which the paraffine has disappeared in one way or another always become centers of absorption for Na_2SO_4, which then readily disintegrates the pottery. In spite of these disturbing elements in the experiment, there can be no question that $CuSO_4$ has

TABLE I

Antagonism Between $CuSO_4$ and Na_2SO_4 For Barley—Adobe Soil, First Crop

No.	% Na_2SO_4 Added	$CuSO_4$ in Parts per Million	Wt. of Straw	Wt. of Grain	Wt Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.5%	100	6.98	8.02	15.00	1.80	16.80
2	.5%	100	5.73	2.27	8.00	1.30	9.30
3	.5%	200	8.50	4.50	13.00	2.70	15.70
4	.5%	200	6.90	4.10	11.00	2.00	13.00
5	.5%	300	7.25	5.95	13.20	1.70	14.90
6	.5%	300	7.70	4.30	12.00	1.00	13.00
7	.5%	400	8.85	5.15	14.00	1.50	15.50
8	.5%	400	9.20	3.60	12.80	2.80	15 60
9	.5%	500	11.60	5.50	17.10	2.30	19.40
10	.5%	500	8.30	5.20	13.50	2.00	15.50
11	.5%	600	8.23	5.27	13.50	2.00	15.50
12	.5%	600	9.15	3.35	12.50	2.50	15.00
13	.5%	700	9.25	5.15	14.40	2.40	16.80
14	.5%	700	6.40	3.60	10.00	1.40	11.40
15	.5%	800	9.20	5.30	14.50	1.90	16.40
16	.5%	800	7.55	3.95	11.50	1.20	12.70
17	.5%	—	7.13	2.17	9.30	.85	10.15
18	.5%	—	7.33	1.07	8.40	1.50	9.90
19	—	—	10.70	—	10.70	2.50	13.20
20	—	—	14.00	4.30	18.30	2.00	20.30
21	—	—	12.05	1.75	14.80	1.40	16.20

exercised a definitely antagonistic effect to the toxicity of Na_2SO_4. Concentrations no greater than 500 parts per million $CuSO_4$ were sufficient in all cases to give the maximum antagonism to .5 percent of Na_2SO_4, and in the second and third crops, which are probably more reliable criteria than the first crop, 100 and 200 parts per million were fully as efficacious, if not more so than the larger amounts. These considerations would seem to indicate that amounts of $CuSO_4$ equivalent to from one tenth to one fiftieth of the amount of Na_2SO_4 present are sufficient to antagonize the latter salt when it is present in soil at concentrations of about .5 percent.

As regards grain yields, the antagonism of $CuSO_4$ to Na_2SO_4 is much more marked than in the case of straw yields. This is particularly so for the first and second crop of the series. In the third crop,

TABLE II

Antagonism Between $CuSO_4$ and Na_2SO_4 For Barley—Adobe Soil, Second Crop

No.	% Na_2SO_4 Added	$CuSO_4$ in Parts per Million	Wt. of Straw.	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.5%	100	2.58	1.00	3.58	.50	4.08
2	.5%	100	2.75	.55	3.30	.55	3.85
3	.5%	200	3.66	1.04	4.70	.52	5.22
4	.5%	200	2.35	.65	3.00	.65	3.65
5	.5%	300	2.70	.80	3.50	.50	4.00
6	.5%	300	2.56	.88	3.44	.38	3.82
7	.5%	400	2.62	.60	3.22	.40	3.62
8	.5%	400	3.77	.75	4.52	.75	5.27
9	.5%	500					
10	.5%	500	2.55	.40	2.95	.60	3.55
11	.5%	600	2.05	.75	2.80	.50	3.30
12	.5%	600	2.25	.45	2.70	.95	3.65
13	.5%	700	3.10	.20	3.30	1.00	4.30
14	.5%	700	3.00	1.00	4.00	.80	4.80
15	.5%	800	2.50	.50	3.00	.50	3.50
16	.5%	800	2.65	.40	3.05	.40	3.45
17	.5%	—	2.61	.35	2.96	.55	3.31
18	.5%	—	2.00	.30	2.30	.70	3.00
19	—	—	2.75	.25	3.00	.25	3.25
20	—	—	2.15	.25	2.40		2.40

TABLE III

Antagonism Between $CuSO_4$ and Na_2SO_4 For Barley—Adobe Soil, Third Crop

No.	% Na_2SO_4 Added	$CuSO_4$ in Parts per Million	Wt. of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.5%	100	18.90	2.10	21.00	2.00	23.00
2	.5%	100	4.90	1.60	6.50	.40	6.90
3	.5%	200	7.60	3.40	11.00	1.24	12.24
4	.5%	200	10.00	2.00	12.00	1.05	13.05
5	.5%	300	13.30	1.80	15.10	.85	15.95
6	.5%	300	5.40	2.60	8.00	.80	8.80
7	.5%	400	4.80	3.80	8.60	1.10	9.70
8	.5%	400	5.70	2.30	8.00	1.16	9.16
9	.5%	500	12.20	3.20	15.40	1.20	16.60
10	.5%	500	7.40	1.60	9.00	1.50	10.50
11	.5%	600	4.30	3.70	8.00	.94	8.94
12	.5%	600	6.10	1.90	8.00	1.00	9.00
13	.5%	700	5.50	3.30	8.80	1.50	10.30
14	.5%	700	5.55	3.50	9.05	.95	10.00
15	.5%	800	6.10	2.80	8.90	1.00	9.90
16	.5%	800	6.00	3.00	9.00	.70	9.70
17	.5%	—	4.40	2.00	6.40	.64	7.04
18	.5%	—	4.00	3.00	7.00	.90	7.90
19	—	—	7.50	2.50	10.00	1.56	11.56
20	—	—	12.70	1.30	14.00	1.87	15.87

the effect is relatively slight. The antagonism is most marked in the first and third crops at concentrations of $CuSO_4$ in excess of 300 parts per million, while in the second crop it is just as marked at 100 parts per million as at 200 and 300 parts per million and much more marked than at the higher concentrations.

Root yields seem to have been definitely improved by the antagonistic influence of $CuSO_4$ to Na_2SO_4 in the first crop. In the second crop, the effect was barely perceptible at the higher concentrations of $CuSO_4$ employed, but it was again clearly evident in the third crop, despite the poor agreement between some of the yields of the duplicate pots.

Series II

$CuCl_2$ versus NaCl—Adobe Soil.

NaCl .3 percent constant—$CuCl_2$ varying.

As was the case in Series I, three consecutive crops were grown and harvested in this series. The antagonism between Cu and Na seem to be very much more marked, however, in Series II than in Series I. The results obtained are given in Tables IV, V, and VI, for the first, second, and third crops, respectively.

As regards straw production in the first crop, increases in yield, due to the antagonistic effect of $CuCl_2$ to NaCl, rise to a maximum of 75 percent over that obtained in the pots treated with NaCl alone. Small additions of 50 to 100 parts per million of $CuCl_2$ seem to have just as strong an antagonizing influence as larger applications of that salt. Additions of $CuCl_2$, equivalent to 300 or 350 parts per million, still show as high antagonizing powers as the smaller amounts. Additions of larger concentrations of $CuCl_2$, however, do not show an antagonizing power; but, even up to and including concentrations of 500 parts per million $CuCl_2$, they do not increase the toxicity of .3 per cent. NaCl. Higher concentrations of $CuCl_2$ than 500 parts per million were not tested. In the second crop, straw production, owing to the unfavorable conditions for growth at the time, was unsatisfactory, but shows clearly enough the antagonism between $CuCl_2$ and NaCl at nearly all concentrations used. This was true, moreover, despite the fact that the toxicity of .3 percent NaCl was scarcely manifest, due apparently to the general poor growing conditions for the crop. In the third crop, the antagonism as regards the straw yields is very marked. The toxicity of NaCl, as shown in Table VI, reduces the yield of barley below that in

the untreated control by approximately 60 percent. But concentrations of $CuCl_2$ with .3 percent NaCl increase the yields again to a point only about 30 percent below the yield of the control plants. Again, as in the first series, all the concentrations of $CuCl_2$ used exhibit antagonizing powers to NaCl, and the smallest concentrations are as effective as the larger ones, if not more so.

TABLE IV

Antagonism Between $CuCl_2$ and NaCl For Barley—Adobe Soil, First Crop

No.	% NaCl Added	$CuCl_2$ Added in Parts per Million	Wt. of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.3%	50	6.28	1.12	7.40	1.75	9.15
2	.3%	50	4.87	2.13	7.00	1.00	8.00
3	.3%	100	7.68	4.32	12.00	1.30	13.30
4	.3%	100	8.00	3.50	11.50	1.00	12.50
5	.3%	150	6.22	3.78	10.00	1.20	11.20
6	.3%	150	6.00	1.80	7.80	.70	8.50
7	.3%	200	6.63	3.37	10.00	1.50	11.50
8	.3%	200	7.40	3.40	10.80	1.70	12.50
9	.3%	250	6.18	2.82	9.00	1.20	10.20
10	.3%	250	6.08	2.52	8.60	2.00	10.60
11	.3%	300	5.98	3.82	9.80	1.40	11.20
12	.3%	300	6.07	1.93	8.00	.40	8.40
13	—	—	—	—	—	—	—
14	.3%	350	6.38	1.82	8.20	.60	8.80
15	.3%	400	5.28	2.22	7.50	1.00	8.50
16	.3%	400	4.20	4.30	8.50	1.20	9.70
17	.3%	450	5.60	1.80	7.40	.70	8.10
18	.3%	450	4.88	2.62	7.50	.40	7.90
19	.3%	500	4.76	2.64	7.40	.70	8.10
20	.3%	500	4.26	3.04	7.30	.65	7.95
21	.3%	—	5.23	.37	5.60	.81	6.41
22	.3%	—	4.08	.92	5.00	.40	5.40
23	—	—	10.70	—	10.70	2.50	13.20
24	—	—	14.00	4.30	18.30	2.00	20.30
25	—	—	12.05	1.75	14.80	1.40	16.20

Very much more marked, however, than the antagonism which characterizes the three series as regards straw yields, is that concerned with the grain yields. In the first crop, the grain yields are from three to six times as great in the copper-treated cultures as in those receiving only NaCl, and equal, and in certain instances surpass, in quantity, the yields of the untreated control soils. In the second crop, the grain yields are only slightly increased through the instrumentality of the antagonism in question. In the third crop, the yields of grain in the antagonism cultures are doubled and even trebled when compared

with those obtained from the cultures treated with NaCl alone and in many instances are equivalent to those obtained from the untreated soil. It is to be noted again that the smaller applications of the copper salt appear to be as effective antagonistic agents as the largest applications.

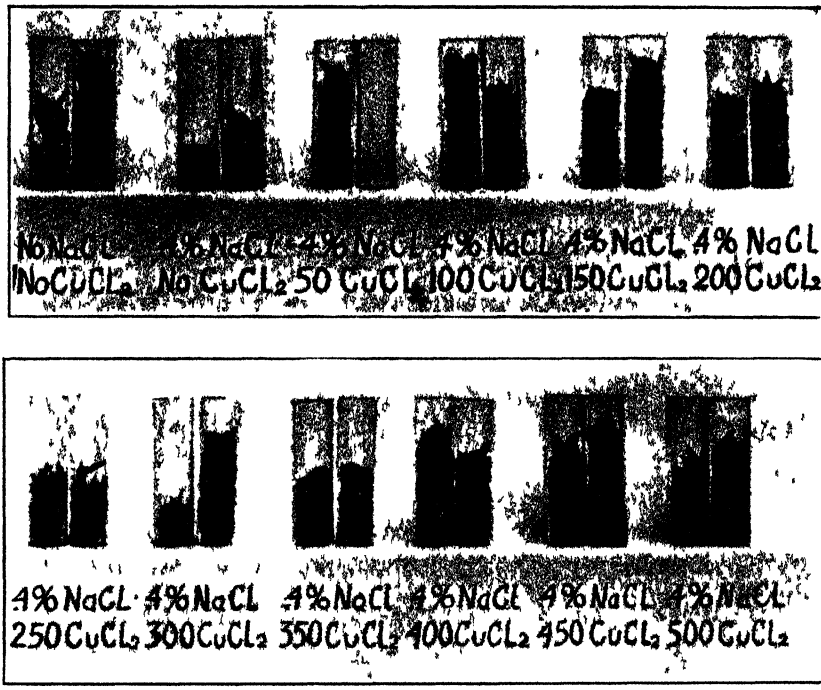


FIG. 1 $CuCl_2$ *vs* NaCl Showing the marked antagonism between the two salts for barley grain yields on the Berkeley adobe soil even to the third crop after one treatment The yield from one of the duplicate pots in the third pair was lost as shown by the empty vial in the photograph

The root yields are very markedly improved in the cultures by addition of $CuCl_2$ to the NaCl in all three crops and particularly so in the cases of the smaller additions of the copper salt.

TABLE V

Antagonism Between $CuCl_2$ and NaCl For Barley—Adobe Soil, Second Crop

No.	% NaCl Added	$CuCl_2$ in Parts per Million	Wt. of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.3%	50	2.88	.88	3.76	.88	4.64
2	.3%	50	2.84	.46	3.30	.50	3.80
3	.3%	100	2.44	.40	2.84	.40	3.24
4	.3%	100	2.13	.45	2.58	.45	3.03
5	.3%	150	3.70	.35	4.05	.44	4.49
6	.3%	150	2.82	.58	3.40	.50	3.90
7	.3%	200	3.75	.65	4.40	.85	5.25
8	.3%	200	3.10	.60	3.70	.60	4.30
9	.3%	250	2.18	.42	2.60	.42	3.02
10	.3%	250	2.65	.95	3.60	.95	4.55
11	.3%	300	2.13	.57	2.70	.27	2.97
12	.3%	300	2.30	.40	2.70	.40	3.10
13	.3%	350	3.00	.80	3.80	.20	4.00
14	.3%	350	2.49	.66	3.15	.66	3.81
15	.3%	400	1.93	.65	2.58	.65	3.23
16	.3%	400	3.72	.18	3.90	.18	4.08
17	.3%	450	2.85	.65	3.50	.65	4.15
18	.3%	450	2.30	.65	2.95	.65	3.60
19	.3%	500	3.10	.85	3.95	.25	4.20
20	.3%	500	2.55	.45	3.00	.45	3.45
21	.3%	—	2.50	.05	2.55	.65	3.20
22	.3%	—	3.10	.95	4.05	.45	4.50
23	—	—	2.75	.25	3.00	.25	3.25
24	—	---	2.15	.25	2.40	.25	2.65

Series III

$CuCO_3$ versus Na_2CO_3—Adobe Soil

Na_2CO_3 .3 percent constant—$CuCO_3$ varying

Only two crops were grown in Series III. Neither as regards straw production nor grain production was there any strong evidence of antagonism between Na_2CO_3 and $CuCO_3$. It did seem, however, that the larger applications of the copper salt used showed a distinct tendency to antagonize the toxic properties of .3 percent Na_2CO_3 in the soil. Contrary to the behavior of the foregoing series, the one here under consideration showed the small amounts of the copper salt to be much less effective than the larger amounts and the evidence seems even to point to an increase of toxicity when the copper salt in low concentrations is added to the sodium salt. These observations hold for the second as well as for the first crop, though the second crop cannot be seriously considered, for the same reasons that made the

TABLE VI

Antagonism Between $CuCl_2$ and NaCl For Barley—Adobe Soil, Third Crop

No.	% NaCl Added	$CuCl_2$ in Parts per Million	Wt. of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.3%	50	6.30	2.70	9.00	1.16	10.16
2	.3%	50	4.50	1.70	6.20	1.40	7.60
3	.3%	100	5.10	2.70	7.80	.74	8.54
4	.3%	100	5.75	1.65	7.40	.40	7.80
5	.3%	150	3.50	2.10	5.60	.25	5.85
6	.3%	150	7.20	2.40	9.60	.50	10.10
7	.3%	200	6.14	1.46	7.60	.40	8.00
8	.3%	200	5.32	2.08	7.40	.62	8.02
9	.3%	250	3.35	1.25	4.60	.58	5.18
10	.3%	250	4.70	1.30	6.00	.36	6.36
11	.3%	300	4.90	.50	5.40	.60	6.00
12	.3%	300	5.50	2.30	7.80	Lost	7.80
13	.3%	350	4.32	1.28	5.60	.20	5.80
14	.3%	350	5.00	1.20	6.20	Lost	6.20
15	.3%	400	4.00	2.00	6.00	Lost	6.00
16	.3%	400	4.20	1.80	6.00	.20	6.20
17	.3%	450	4.40	2.00	6.40	.35	6.75
18	.3%	450	4.50	2.50	7.00	.31	7.31
19	.3%	500	3.60	1.40	5.00	.38	5.38
20	.3%	500	3.85	2.35	6.20	.28	6.48
21	.3%	—	4.10	.60	4.70	.65	5.35
22	.3%	—	3.04	1.16	4.20	.22	4.42
23	—	—	7.50	2.50	10.00	1.56	11.56
24	—	—	12.70	1.30	14.00	1.87	15.87

second crop in the other series an uncertain factor. Root yields were not obtained at all, owing to the bad physical condition of the adobe soil, induced by the Na_2CO_3 applications. Unfortunately, this series was not continued through the third crop as were the others and the conclusions are, consequently, of less value than the foregoing.

Series IV

$ZnSO_4$ versus Na_2SO_4—Adobe Soil

Na_2SO_4 .6 percent constant—$ZnSO_4$ varying

Owing to the difficulty encountered with the creeping of Na_2SO_4 up and out of the pots employed in these experiments, it was decided to try one series with zinc and sodium sulphates in large wide-mouth bottles. The results obtained with this series, which obviated the loss of Na_2SO_4 from the soil, together with the general arrangement of the cultures are given in Table VII. Control cultures in bottles

with no salt treatment were inadvertently omitted from the series. This omission in the experiment is regrettable, but owing to the definiteness of the toxic effects of Na_2SO_4 obtained and to the equally definite evidences of antagonism between zinc and sodium, it does not militate seriously against the usefulness and significance of the results.

TABLE VII

Antagonism Between $ZnSo_4$ and Na_2SO_4 For Barley—Adobe Soil, One Crop

No.	% Na_2SO_4 Added	$ZnSO_4$ in Parts per Million	Wt. of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots
			g.	g.	g.	
1	.6%	—	5.30	2.10	7.40	Did not harvest roots. Could not get them out of bottles.
2	.6%	—	5.60	2.00	7.60	
3	.6%	100	5.20	4.00	9.20	
4	.6%	100	5.80	2.80	8.60	
5	.6%	300	7.10	4.90	12.00	
6	.6%	300	5.40	2.20	7.60	
7	.6%	500	6.00	4.00	10.40	
8	.6%	500	7.30	3.30	10.60	
9	.6%	700	11.30	4.50	15.80	
10	.6%	700	7.70	3.80	11.50	
11	.6%	1,000	12.60	2.80	15.40	
12	.6%	1,000	12.50	4.30	16.80	

The straw yields are clearly influenced for the better by the applications of $ZnSO_4$ to the Na_2SO_4-treated soil. Particularly is this true of cultures receiving the larger applications of $ZnSO_4$. There can be no doubt of the definite antagonism indicated in these data. In the case of the grain yields likewise, the evidences of antagonism are very clear, but the smaller concentrations of $ZnSO_4$ appear to have been as effective in antagonism as regards grain production as the larger concentrations of that salt. It was found impossible to remove the soil from the bottles at the end of the experiment in such a fashion as to permit of the determination of root yields. Hence the latter are not given in the table. It is to be noted in connection with Series IV that the agreement between duplicate cultures is much better in bottles as containers than in pots. Particularly when alkali salts are involved, the use of bottles or similar glass containers would seem to deserve preference over even paraffined pots. Whether or not the ordinary glazed crocks now employed by us will combine the advantages of the glass with the advantages of earthenware pots will, we hop bee, soon determined.

Series V

$ZnCl_2$ versus NaCl—Adobe Soil

NaCl .4 percent constant—$ZnCl_2$ varying

Table VIII shows the arrangement of the cultures in this series and gives the concentrations of salts used. Despite poor agreement between the yields of duplicate pots, it is clearly shown in the table that $ZnCl_2$ exercises a powerful antagonistic effect to the toxic proper-

TABLE VIII

Antagonism Between $ZnCl_2$ and NaCl For Barley—Adobe Soil, One Crop

No.	% NaCl Added	$ZnCl_2$ in Parts per Million	Wt. of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt of Roots	Wt of Total Dry Matter
			g.	g.	g.	g.	g.
1	.4%	50	4.50	2.30	6.80	.40	7.20
2	.4%	50	7.30	3.70	11.00	.60	11.60
3	.4%	100	6.70	2 90	9.60	1.20	10.80
4	.4%	100	6.82	2.18	9.00	.75	9.75
5	.4%	300	9.20	4.80	14.00	.75	14.75
6	.4%	300	7.92	4.48	12.40	.80	13.20
7	.4%	500	6.70	3.70	10.40	1.00	11.40
8	.4%	500	7.16	2.34	9.50	.96	10.46
9	.4%	700	6.24	3.16	9.40	1.34	10 74
10	.4%	700	5.75	2.75	8.50	.46	8.96
11	.4%	—	5.00	1 90	6.90	.40	7.30
12	.4%	—	4.70	2.30	7.00	.55	7.55
13	.4%	—	5.75	.75	6.50	.45	6.95
14	—	—	12.20	5.30	17.50	2.50	20.00
15	—	—	8.44	4.56	13.00	3.40	16.40

ties of NaCl, in so far as the production of total dry matter of barley plants is concerned. As regards grain and root production, the antagonistic effect mentioned is not so marked, but is distinct and great enough to satisfy the most critical of its existence and potency. The poor agreement between the yields of duplicate pots, which has been referred to above, does not permit of an exact appraisal of the relative efficiencies of small and large amounts of $ZnCl_2$ as antagonistic agents to the toxic effects of .4 percent of NaCl. Moreover, 50 parts per million $ZnCl_2$, the lowest concentration of that salt employed, seems to be of high potency in the direction indicated. Nevertheless, concentrations of 300 parts per million $ZnCl_2$ seem to be considerably more efficacious than either smaller or larger concentrations of that salt. Only one crop was grown in the pots of this series.

Series VI

$ZnSO_4$ versus NaCl—Adobe Soil

NaCl .4 percent constant—$ZnSO_4$ varying

This series brings into play four ions instead of three, as in the series in which the antagonizing salts possess the same anion. The results are given in Table IX, together with the usual explanatory data. Once again, we see the marked evidences of the toxic properties of .4 percent NaCl to barley in the adobe soil and the equally marked

TABLE IX

Antagonism Between $ZnSO_4$ and NaCl For Barley—Adobe Soil, One Crop

No.	% NaCl Added	$ZnSO_4$ in Parts per Million	Wt. of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.4%	50	5.88	3.72	9.60	.65	10.25
2	.4%	50	7.90	3.80	11.70	.65	12.35
3	.4%	100	8.90	3.70	12.60	1.10	13.70
4	.4%	100	9.96	4.24	14.20	1.14	15.34
5	.4%	300	8.80	5.00	13.80	.40	14.20
6	.4%	300	9.04	4.56	13.60	.75	.75
7	.4%	500	5.24	4.16	9.40	.84	10.24
8	.4%	500	6.10	3.50	9.60	.70	10.30
9	.4%	700	7.30	4.50	11.80	1.00	12.80
10	.4%	700	7.30	2.70	10.00	.55	10.55
11	.4%	1,000	8.15	3.85	12.00	.80	12.80
12	.4%	1,000	6.30	3.70	10.00	1.20	11.20
13	.4%	——	5.00	1.90	6.90	.40	7.30
14	.4%	——	4.70	2.30	7.00	.55	7.55
15	.4%	——	5.75	.75	6.50	.45	6.95
16	——	——	12.20	5.30	17.50	2.50	20.00
17	——	——	8.44	4.56	13.00	3.40	16.40

antagonizing properties thereto of another salt. As triplicate pots show clearly, less than half the yield of barley is obtained in the NaCl-treated soils of that produced in the control pots. The addition to the NaCl, however, of 50 parts per million of $ZnSO_4$ very largely overcomes the toxic effect in question, and the addition of 100, or 300 parts per million of $ZnSO_4$ almost entirely obliterates it. The addition of larger quantities of $ZnSO_4$ seems to be less effective than the last two named, but about as effective as 50 parts per million up to and including the largest quantity used, viz., 1,000 parts per million. Again, the effects of antagonism are marked with respect to grain and straw production, as well as with respect to root yields, though perhaps least striking in the latter case.

Series VII

$CuSO_4$ versus Na_2SO_4—Oakley Soil

Na_2SO_4 .5 percent constant—$CuSO_4$ varying

Two errors were made in this series. The first consisted in the omission of control untreated pots from the experiment. The second was the addition of equal amounts of ammonium nitrate to all pots in

TABLE X*

Antagonism Between $CuSO_4$ and Na_2SO_4 For Barley—Oakley Soil, One Crop

No.	% Na_2SO_4 Added	$CuSO_4$ in Parts per Million	Wt. of Straw	Wt of Grain	Wt Dry Matter Above Surface	Wt of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.5%	100	8.00	2.00	10.00	.55	10.55
2	.5%	100	8.38	3.62	12.00	.67	12.67
3	.5%	200	10.45	2.55	13.00	.50	13.50
4	.5%	200	9.27	3.43	12.70	.80	13.50
5	.5%	300	8.50	2.00	10.50	.55	11.05
6	.5%	300	10.62	5.18	15.80	.50	16.30
7	.5%	400	7.80	2.70	10.50	.50	11.00
8	.5%	400	6.60	1.40	8.00	.23	8.23
9	.5%	—	6.05	2.95	9.00	.58	9.58
10	.5%	—	7.90	2.10	10.00	.90	10.90

* 1 g. NH_4NO_3 added to each pot 1 month after plants were up.

order to obtain better absolute yields. The reasons for referring to these as errors are obvious. Nevertheless, the data in Table X are interesting, inasmuch as they do indicate, in spite of the presence of ammonium nitrate in the pots, distinct antagonism between Na_2SO_4 and $CuSO_4$.

Series VIII

$ZnCl_2$ versus NaCl—Oakley Soil

NaCl .4 percent constant—$ZnCl_2$ varying

Despite the errors of the two series just described, evidence on the existence of antagonism between the heavy metals and the alkali salts in the Oakley soil is to be found in Table XI of Series VIII, in which, moreover, the larger concentration of NaCl employed made possible the bringing into stronger relief the antagonisms in question. The data in Table XI speak largely for themselves. It remains but to mention that the higher concentration of $ZnCl_2$ used inhibited the growth of barley entirely in this series and that grain was produced only in the cultures in which the most marked antagonism occurred.

TABLE XI

Antagonism Between $ZnCl_2$ and NaCl For Barley—Oakley Soil, One Crop

No.	% NaCl Added	$ZnCl_2$ in Parts per Million	Wt of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.4%	100	3.85	.05	3.90	.23	4.30
2	.4%	100	2.10	—	2.10	.17	2.27
3	.4%	200	4.80	.65	5.45	.32	5.77
4	.4%	200	2.50	.50	3.00	.60	3.60
5	.4%	300	2.05	—	2.05	.35	2.40
6	.4%	300	1.55	—	1.55	.15	1.70
7	.4%	400	1.10	—	1.10	.08	1.18
8	.4%	400	—	—	—	—	—
9	.4%	500	—	—	—	—	—
10	.4%	500	—	—	—	—	—
11	.4%	1,000	—	—	—	—	—
12	.4%	1,000	—	—	—	—	—
13	.4%	—	1.60	—	1.60	.17	1.77
14	.4%	—	1.65	—	1.65	.24	1.89
15	—	—	3.25	—	3.25	.32	3.57
16	—	—	2.97	—	2.97	.24	3.14

TABLE XII

Antagonism Between $CuSO_4$ and NaCl For Barley—Oakley Soil, One Crop

No.	% NaCl Added	$CuSO_4$ in Parts per Million	Wt. of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.4%	50	24.60	4.20	28.80	.78	29.58
2	.4%	50	13.90	6.10	20.00	.80	20.80
3	.4%	100	20.90	2.10	23.00	.90	23.90
4	.4%	100	18.80	1.20	20.00	.76	20.76
5	.4%	200	20.40	2.40	22.80	.40	23.20
6	.4%	200	13.00	3.20	16.20	Lost	16.20
7	.4%	300	29.40	1.00	30.40	.80	31.20
8	.4%	300	13.40	1.60	15.00	—	15.00
9	.4%	400	16.50	1.00	17.50	.40	17.90
10	.4%	400	9.80	3.20	13.00	1.56	14.56
11	.4%	500	15.30	1.70	17.00	.52	17.52
12	.4%	500	11.90	1.30	13.20	.40	13.60
13	.4%	—	8.10	1.90	10.00	.16	10.16
14	.4%	—	12.20	2.80	15.00	.40	15.40
15	—	—	20.40	2.40	22.80	2.60	25.40
16	—	—	24.00	2.00	26.00	1.00	27.00
7	—	—	12.80	4.20	17.00	1.20	18.20

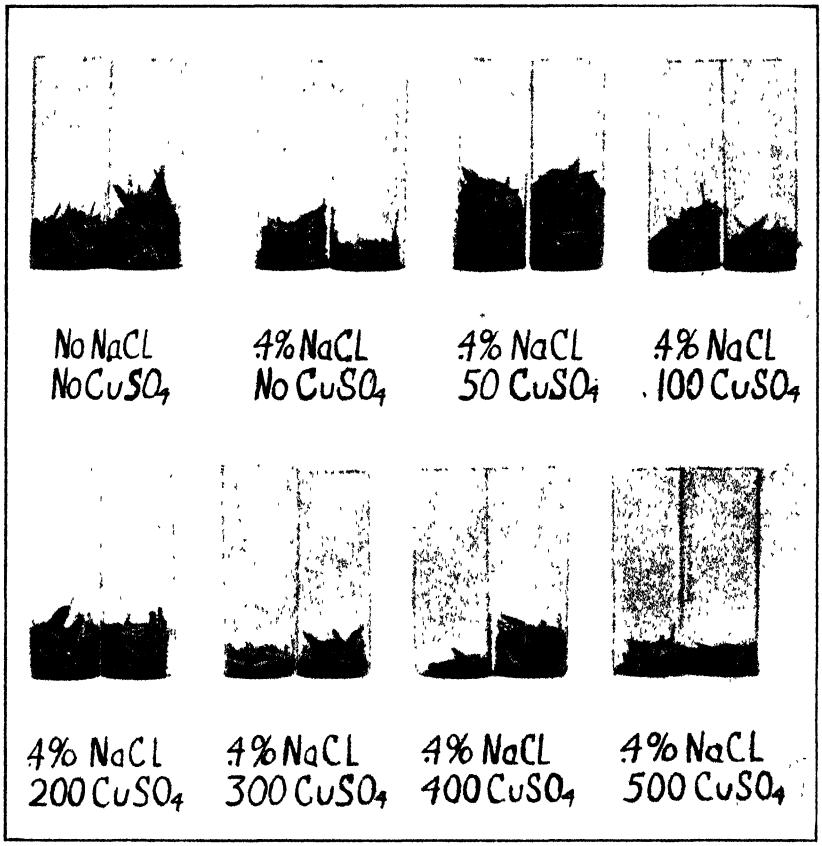


FIG. 2. $CuSO_4$ *vs.* NaCl. Showing yields of grain from Oakley soil, first crop with and without different salt treatment. The duplicate vials represent the yields of duplicate pots and give an idea of the individual variability in plant production. The antagonism obtaining here is clearly very marked.

Series IX

$CuSO_4$ versus NaCl—Oakley Soil

NaCl .4 percent constant—$CuSO_4$ varying

Table XII gives the results obtained and other necessary data in regard to Series IX. The Oakley soil used in this case was different from any of the lots used in the other series described in this paper and hence gave very different yields in all the pots. The figures submitted

show, as clearly as any obtained with the adobe soil, how markedly $CuSO_4$ antagonizes NaCl. Even 50 parts per million of $CuSO_4$ added to .4 percent NaCl is sufficient to obliterate entirely the toxic effects of the last-named salt and perhaps even to go beyond in the direction of stimulation. Large quantities of $CuSO_4$ as high as 400 and 500 parts per million are also very effective in antagonizing .4 percent NaCl. There would seem to be much promise in the data obtained for application to alkali conditions in the field, like those obtaining in the Imperial Valley.

Series X

$ZnSO_4$ versus NaCl—Oakley Soil

NaCl .4 percent constant—$ZnSO_4$ varying

While the absolute yields in this series were small, the data in Table XIII show clearly that $ZnSO_4$ has a definite power of antagonizing NaCl when the latter is used at the toxic concentration of .4 percent. At high concentrations of $ZnSO_4$ plus .4 percent NaCl, no growth was obtained.

TABLE XIII

Antagonism Between $ZnSO_4$ and NaCl For Barley—Oakley Soil, First Crop

No.	% NaCl Added	$ZnSO_4$ in Parts per Million	Wt. of Straw	Wt. of Grain	Wt. Dry Matter Above Surface	Wt. of Roots	Wt. of Total Dry Matter
			g.	g.	g.	g.	g.
1	.4%	100	1.73	.17	1.90	.20	2.10
2	.4%	100	3.85	—	3.85	.70	4.55
3	.4%	200	2.40	.30	2.70	.52	3.22
4	.4%	200	2.42	.18	2.60	.70	3.30
5	.4%	300	3.70	—	3.70	.45	4.15
6	.4%	300	1.95	.05	2.00	.40	2.40
7	.4%	400	2.15	—	2.15	.35	2.50
8	.4%	400	1.90	.10	2.00	.34	2.34
9	.4%	500	3.35	—	3.35	.48	3.83
10	.4%	500	1.67	.08	1.75	.22	1.97
11	.4%	1,000	Trace	—	Trace	—	—
12	.4%	1,000	Trace	—	Trace	—	—
13	.4%	2,000	—	—	—	—	—
14	.4%	2,000	—	—	—	—	—
15	.4%	3,000	—	—	—	—	—
16	.4%	3,000	—	—	—	—	—
17	.4%	—	.72	—	.72	.06	.78
18	.4%	—	1.05	—	1.05	.10	1.15
19	.4%	—	.75	—	.75	.05	.80
20	—	—	4.70	—	4.70	.25	4.95
21	—	—	3.30	—	3.30	.32	3.62

General Discussion

It may be stated without qualification that the data submitted above are evidence of the antagonistic action of the heavy metals to alkali salts for crop plants grown in pots. Moreover, our evidence appears to be the first of the kind ever published. If, as now seems likely, the principles thus adduced may be applied to field conditions, a new factor of safety may be introduced into alkali problems which may possess major importance in competent hands. From the scientific standpoint, on the other hand, the facts which we have obtained are equally interesting and important and indicate a field of investigation of great promise with regard to the mechanism of the antagonistic action which we have noted.

That the effects noted are, in a sense, certainly not ephemeral ones may be gleaned from the data submitted for the adobe soil in which three crops were grown in succession in some of the series and antagonism was shown to obtain in all cases. It is unfortunate that similar results were not obtained for the Oakley soil which could be submitted in this paper, but the results of certain series which were not complete and therefore could not be given here indicate, as one would expect, that the facts adduced in the case of the adobe soil are of equal cogency in their application to the Oakley soil.

Other general features of our experiments, which may demand special attention here, are the following: The small quantities of the metals which are sufficient to antagonize large quantities of alkali salts render the economics of the applications of the scientific principles involved fairly simple. If it should prove possible to employ refuse from metallic ores for the purpose, the task of antagonizing the alkali salts in soils should prove particularly simple. The fact, also, that zinc is nearly as effective as copper in the direction noted may be indicative of possibilities in the same line with other and cheaper metals, a point which we shall hope to determine in future experiments.

The reproductions of photographs of some of the grain yields in vials as containers will serve to emphasize the data for the yields which are given in the tables.

In the discussions given herewith, the authors have been fully cognizant of the differences which obtain between the amounts of salts applied to the soil and those which remain actively in solution in the soil water. We have not attempted, therefore, to give in the tables

any idea as to the actual amounts of interacting salts in the antagonisms noted. For one thing, this would be impossible with the methods now possessed by soil investigators. Besides, we do not consider our results as applying to any phase of the problem except that of the actual conditions which exist in soils when certain amounts of the alkali salts are present, and when their effects are more or less modified by the addition of other salts. In view of these considerations, it appears that the question raised by us, in anticipation of its being brought forward by others, is of little pertinence in so far as our main thesis is concerned.

SUMMARY

Experiments bearing on the antagonism between salts of the heavy metals, Cu and Zn, and the common alkali salts of soils have been carried out as follows: Plants were grown in pots and two different soils were tested. NaCl, Na_2SO_4 and Na_2CO_3 were used in toxic and constant quantities, the salts of the heavy metals varying in quantity within a given series. Barley was the plant grown. Briefly, the following results were obtained:

1. Copper and zinc antagonize NaCl, Na_2SO_4 and Na_2CO_3 in the Berkeley adobe soil, and the antagonism is evident even if three successive crops are used as criteria, and when only the metallic ions vary.

2. When four ions are introduced, for example, as in the case of $CuSO_4$ versus NaCl, fully as much and even more antagonism is manifest between the heavy metals and the alkali salts.

3. Although only one crop was grown on the Oakley sand, similar evidences of marked antagonism between the heavy metals and the alkali salts were noted. The evidence in this case was, however, particularly striking in the case of $CuSO_4$ versus NaCl.

4. These findings should possess considerable significance in the field reclamation of alkali lands, and particularly in the case of those which do not contain large enough quantities of salts to render them unfit for plant growth by reasons of high osmotic pressures in their soil solutions.

THE EFFECT OF TOBACCO SMOKE AND OF METHYL IODIDE VAPOR ON THE GROWTH OF CERTAIN MICROORGANISMS*

C. A. LUDWIG

During the winter of 1916–'17 the writer of this paper was engaged in a study of the effect of illuminating gas and its constituents on certain bacteria and fungi (3) and as an accompaniment to this work carried out some similar experiments with tobacco smoke and methyl iodide vapor. Since there is no immediate opportunity to carry these experiments further it has seemed desirable to report briefly on them, although the results attained are necessarily quite preliminary in character. The work was done under the direction of Prof. F. C. Newcombe, for whose help the writer wishes here to extend thanks.

TOBACCO SMOKE

Not much experimental work has been reported showing the effect of smoke on bacteria or fungi, although Tassinari (5) showed as long ago as 1888 that tobacco smoke has a retarding effect on a number of pathogenic and non-pathogenic bacteria when they are exposed to the smoke before being put into the sterile nutrient medium. More recently Molisch (4) has shown that tobacco smoke will stop the movements of *Chromatium vinosum* (Ehrenb.) Winogradsky, *Beggiatoa* sp., and *Spirillum* sp.; and that it will retard the growth of *Phycomyces nitens*.

It is quite impossible, of course, to get any accurate idea as to the composition of any sample of smoke without making an analysis of the sample. A number of papers have been written, however, having an especial bearing on a qualitative determination of the compounds present. No attempt will be made here to summarize this work further than to enumerate some of the compounds found and thus to call attention to the complexity of the mixture called smoke. Vohl and Eulenberg (7) reported a series of hydrocarbons of the benzene series or one analogous to it, and in addition formic, propionic, butyric,

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valerianic, and carbonic acids, creosote, ammonium chloride, ammonia, pyridine, picoline, lutidine, collidine, parvoline, coridine, and rubidine. Kissling (2) reported that the strongly poisonous materials (to man) are carbon monoxide, hydrogen sulphide, hydrocyanic acid, picoline bases and nicotine. To this list of compounds in tobacco smoke Thoms (6) added a phenol boiling at 190–200°, furfural (in small amounts), and a substance boiling at 200–260° containing sulphur and nitrogen and no terpenes. Crocker and Knight (1, p. 346) have called attention to the presence of ethylene and correlated its presence with the effect of smoke on some phanerogams.

For exact data as to the cultures used and the methods of conducting the experiments reported below the reader is referred to the writer's former paper (3) in which the influence of illuminating gas and its constituents is discussed. For the purpose of filling the culture chamber with smoke the tubulature of the bell jar was fitted with a two-hole rubber stopper carrying two glass tubes such that one extended very little below the stopper while the other extended well toward the bottom of the chamber. The short tube was then connected to an aspirator and the other to a cob pipe. The pipe was filled with tobacco, "Prince Albert" brand, the aspirator started and the tobacco lighted. When the chamber became filled with a white, opaque smoke cloud, the aspirator was stopped and the tubes plugged. The air in the chamber soon became clear, but the upper surface of everything within, and to some extent the vertical surfaces also, became stained brown. The material producing the brown stain did not extend into the test tubes, and consequently not to the agar, because it was clearly limited to the surface of the cotton plugs. No reactions, therefore, can be laid to these products except as they may have been somewhat volatile and therefore capable of diffusing into the tubes. In some of the tests the smoke was passed through one or two wash bottles containing water. In these cases small amounts of a brown oily substance condensed and floated on the surface of the water, and it took longer to produce the opaque cloud in the chamber.

The development of the bacteria in the smoke was rather variable, perhaps owing to an unavoidable lack of uniformity of the conditions in the different trials. There was a strong tendency for the colony development to begin at the bottom of the slant and progress upward after a preliminary period of no growth. No reason can be given for the very pronounced nature of this tendency in smoke. It was ob-

served, however, that a general but less pronounced tendency for this sort of behavior existed for the cultures in the other gases; and this would seem to indicate that the greater thickness and perhaps moisture content of the substratum at the bottom of the slant together with the heavier inoculation at the beginning of the streak may have had something to do with the results. Apparently the complete prevention of growth at the outset would preclude any assumption that the gases did not diffuse to the bottom of the tubes.

The reports for the following eight organisms are based on two trials in unwashed and one in washed smoke.

Bacillus subtilis.—In the first test there was no visible development in unwashed smoke until the sixth day; in the second test it became visible on the second day. The retardation continued throughout the duration of the 7-day exposure, however, with the washed smoke showing the smaller inhibitive effect. The length of duration of the retarding effect is a point of some significance, as under some circumstances a bacterial culture will show an initial retardation, but will reach a stage of development quite indistinguishable from that of the check within 3 or 4 days.

Bacillus pyocyaneus.—In the unwashed smoke, both trials, the growth of the organism became visible in one day; but the colony had not entirely reached the top of the slant at the end of the exposure.

Bacillus Kieliensis.—In the first trial the colony of *B. Kieliensis* had just become visible in 6 days. In the second trial it had become visible in 2 days in both raw and washed smoke. By the end of the 7-day period, however, it had developed upon little more than half the length of the slant in raw smoke but over the entire length in washed smoke.

Bacillus rubidus.—In these tests the culture of *B. rubidus* was made on potato, and there was no test in washed smoke. In the tests with raw smoke the cultures required 2 days and 4 days respectively to become visible, while the check in air was visible the day after inoculation in both cases.

Sarcina lutea.—The development of *S. lutea* was hindered by the smoke, more so by that which was untreated than by that which was bubbled through water; but in all cases the colony occupied all of the inoculated area and had produced an abundance of material by the close of the 7-day exposure.

Oidium lactis.—The development of *O. lactis* began promptly in

smoke at the bottom of the slant and progressed gradually upward. The colony in the washed smoke covered the surface of the agar in considerably less time than that required by the one in raw smoke.

Cryptococcus Ludwigi.—In neither trial did the colony of this yeast in the raw smoke reach the top of the agar slant by the end of the exposure, although the colony in the treated smoke did reach that degree of development. As with most of the other organisms, the development proceeded from the bottom upwards.

Penicillium stoloniferum.—The development of this green mould was retarded in both the treated and untreated smoke. Conidia were produced normally. By the end of the 7-day period the colony had reached the top of the slant in washed smoke while in the unwashed smoke only the lower half was covered.

The results with the following eight organisms are based on two trials with washed smoke and the same number with unwashed smoke.

Bacterium stewarti.—With *B. stewarti* the washed smoke, as usual, was less toxic than the unwashed. In the test with untreated smoke the area in which the colony had developed to visibility was still limited to a rather small area at the base of the slant at the end of the 6-day exposure.

Bacillus carotovorus.—The colony of *B. carotovorus* in raw smoke had developed on ½ to ¾ of the agar slant by the end of a week. In washed smoke, however, it had extended the entire length of the slant in 4 and 6 days respectively.

Bacillus melonis.—The colony of *B. melonis* was visible within a day after inoculation in washed smoke, but it required 2 days and 4 days respectively to become visible in the other. In the latter condition, also, the colony had not reached the top of the slant at the end of 6 days in either trial.

Bacillus campestris.—In the first test with raw smoke it took 2 days for the colony of *B. campestris* to become visible and it had progressed upward only about 1 cm. at the end of 6 days. In the second test for some unknown reason the culture failed to grow at all. In the treated smoke the colony was visible in one day and it had reached the top of the slant in 6 days.

Bacterium tumefaciens.—The colony of *B. tumefaciens* in untreated smoke did not become visible before 2 days in the more vigorous culture of the two, and in both cases was still confined pretty closely to the base of the slant at the close of the 6-day period. The cultures in the washed smoke grew more vigorously.

Bacillus solanisaprus.—The presence of smoke, either washed or unwashed, proved to be a hindrance to the growth of this organism, with the washed smoke, as usual, exhibiting the less toxicity.

Pseudomonas radicicola.—There was little difference between the behavior of *Ps. radicicola* and of the other bacteria in smoke. In the first trial the colony was still confined to the lower half of the slant in the raw smoke at the end of the exposure, while in the second no growth had taken place. In the treated smoke the growth was more vigorous and the colony extended the entire length of the slant.

Bacillus mycoides.—In the case of *B. mycoides* there was in one case no development in raw smoke and in the other the colony was pretty closely confined to the base of the slant. In washed smoke the colonies had spread nearly or quite over the surface of the agar by the time the experiment ended.

It should perhaps be remarked here in connection with the cultures mentioned above that whether specific mention has been made of the check cultures in air or not, it is to be understood that they were made, that they grew promptly (visible within a day), and that they grew over the entire length of the slant.

It thus appears that tobacco smoke is more or less toxic to the organisms used, although it does not seem to exert such extreme toxicity to them as it does to some phanerogams. In view of the very complicated and variable mixture of compounds which constitute smoke, it is hardly worth while to venture an opinion as to which substance or group of substances exerts the toxic influence. It may not be beside the mark, however, to call attention to the fact that the washed smoke was uniformly less toxic in the tests than was the unwashed smoke, and to suggest that something capable either of being condensed or of being dissolved in water has some part in inducing the reactions.

Methyl-Iodide Vapor

The use of methyl-iodide vapor as a test gas for organisms was undertaken as a test of the reliability of some results obtained from methane prepared from methyl iodide by means of the copper-zinc couple; as it was feared that the gas so produced contained some undecomposed methyl-iodide vapor. In the experiments with methyl iodide the chemical was introduced into the culture chamber by dropping the liquid on a bit of absorbent cotton supported by a glass rod

passing through a rubber stopper. The stopper was immediately put in place in the tubulature of the bell jar which served as the chamber. The liquid then evaporated and diffused to all parts of the chamber, as was amply evidenced by the effects on the cultures. The volume of the jar was approximately 3.8 liters.

In the first trial no measure was secured of the amount of methyl iodide used; but it was considerably more than in the second, where the amount was limited to 5 drops. The results with the different species used were so nearly alike that they will not be discussed separately. The species tested were *Bacillus subtilis*, *B. pyocyaneus*, *B. Kieliensis*, *B. rubidus*, *Sarcina lutea*, *Oidium lactis*, *Cryptococcus Ludwigi*, and *Penicillium stoloniferum*.

Without exception the culture was killed in the test with the larger amount of methyl iodide, *i. e.*, there was no development during a 7-day exposure nor within a period of 24 days after. With the smaller amount of the chemical the development was nearly normal in all cases except that there was a slight slowing down of the growth, capable of detection, however, only for periods of 1 to 4 days. In addition, the pink yeast was slightly pale in color and *B. rubidus* (on potato) was clear yellow instead of orange yellow.

In the third test six drops of the liquid were used and in the fourth something more than ten. The organisms used in these two tests were *Bacterium stewarti*, *B. tumefaciens*, *Bacillus carotovorus*, *B. melonis*, *B. campestris*, *B. solanisaprus*, *B. mycoides*, and *Pseudomonas radicicola*.

The development was uniformly greatly inhibited at first but soon began to proceed rapidly so that in the first experiment the treated cultures overtook the checks in about 4 or 5 days on the average. In the second trial, in which the amount of the chemical was doubled or more than doubled, the inhibiting effect was more permanent. Thus *Bacillus melonis*, *B. solanisaprus*, and *Bacterium tumefaciens* were the only ones to recover and develop as fully as in the air by the close of a 6-day exposure. *Bacillus mycoides* would probably have shown an equal ability had inoculation been by means of a streak, as the development near the point of inoculation (the center of the slant only) did show such an ability. The organism, however, showed a reduction in the power to invade the surface of the substratum in the vapor.

The general effect of methyl-iodide vapor on the organisms tested therefore, as shown by the data presented above, is to induce an initial

great retardation of development followed later by a very vigorous growth unless the amount of the vapor be sufficient to sterilize the inoculated medium, in which case, of course, no development at all follows.

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THE VERTICAL DISTRIBUTION OF VOLVOX IN THE PLANKTON OF LAKE MONONA

GILBERT MORGAN SMITH

On the morning of July 6, 1916, Messrs. Birge and Juday, of the Wisconsin Geological and Natural History Survey, noted a very peculiar condition, while collecting plankton samples from Lake Monona, Madison, Wisconsin. They found that there was a very decided stratum of Volvox colonies at a depth of three meters, while above and below this stratum the colonies were very scarce. Mr. Juday called my attention to this condition, and suggested that I make plankton catches at various times of the day to see if the position of the belt changed. Although the data presented herewith are, at best, fragmentary, they seem worthy of record since nothing is known concerning the vertical distribution of Volvox as a limnetic organism. The "bloom" of this alga is so sporadic that it may be some time before so favorable a condition for a study of this kind again presents itself.

The ordinary method of pumping a measured amount of water (ten liters) from a desired depth and then straining through a filter net was used for collecting the samples, which were then preserved in alcohol until counted. In counting, the volume of the catch was reduced to ten cubic centimeters, then a one-cubic-centimeter sample was taken, put in a trough, and the number of colonies counted through a binocular microscope.

The station where the alga was first discovered is about a kilometer northwest of Winnequah point, at the deepest part of the lake. My samples were collected about a half kilometer out from the Wirka Boat Livery where the water is between ten and thirteen meters deep. This station is about a kilometer and a half from the deepest part of the lake. Sunset occurred at 7:40 on July 6 and the sample from the surface was taken at 7:45. Since it takes about five minutes to adjust the hose, pump the water, label and preserve the sample, the interval between two catches is about five minutes. The same order was followed in every case, the first sample taken at the top and others on down at intervals of a meter. Twilight lasts about an hour at this

season so that it had been dark for nearly an hour when the next series of samples was collected at 9:20 P.M. The following morning the sun rose at 4:26 and the first catch was made at 4:25. A series of samples was collected every succeeding hour until 8:30 and then at 10:00 and again at noon. Collecting was resumed at 5:30 A.M. the following day and continued at hourly intervals until 9:30 A.M. when the sky became cloudy and a sharp squall blew up. The wind caused considerable wave action, so that what vertical distribution there had been was disturbed. The results of these collections are shown in the following table. Those of the last day's collecting are not incorporated since practically no colonies were found.

TABLE I

Number of Colonies per Liter at the Various Depths

Depth in Meters	July 6 (Evening)		July 7 (Morning)						
	7 45	9 20	4 25	5 30	6:30	7 30	8 30	10 00	12 00
0	890	1,900	670	11,500	10,100	8,600	13,800	14,300	28,100
1	1,200	820	10,810	12,400	15,500	24,500	19,300	13,300	14,600
2	4,390	1,620	5,840	17,500	5,800	12,500	10,900	5,230	2,100
3	33,200	2,300	9,600	7,700	1,670	2,130	1,280	3,560	980
4	2,790	11,300	3,150	1,290	650	590	1,250	840	510
5	—	9,010	810	—	—	—	—	—	—
6	—	3 980	—	—	—	—	—	—	—

The weather conditions, at the time the collections were made, are important factors. Had there been any wind stirring up the water the vertical distribution would have been affected. Since the 6th and 7th were exceptionally calm days this factor may be neglected. The following meteorological data have been furnished by Mr. E. R. Miller, the Local Forecaster of the Weather Bureau. The meteorological observations are taken at a point about forty-five meters above the level of the lake where there is always a greater air movement than on the lake surface. Shaw (8) gives methods of estimating the velocity of the wind so that the weather conditions of the 6th and the 7th may be easily visualized.[1] Both of these days were bright and sunny, the

[1] When the wind is blowing at two miles per hour the direction of wind is shown by smoke drift, but not by wind vanes; at five miles per hour the wind is felt on the face, leaves rustle, and ordinary weather vanes move; while at ten miles per hour leaves and small twigs are in constant motion and the wind extends a small flag (p. 31).

amount of sunshine being very near the maximum for this time of the year. The local office of the Weather Bureau records the total energy of the sun's rays in the form of calories per minute per square centimeter of surface. The curves for the two days are almost identical, so that no distinction can be made between the two on the basis of different light intensities. Table II gives the light intensities at the times that the samples were collected on the 7th of July.

TABLE II

Weather Conditions at the Time Samples Were Collected

Velocity of Wind			Total Sunshine (Morning July 7)	
Date	Av. Vel. in Miles per Hour	Max. Vel.	Hour	Cal. per Min. per Sq. Cm.
July 6.....	3.6	8	4:25	0.00
			5:30	0.12
July 7.	4.2	8	6:30	0.39
			7:30	0.60
			8:30	0.82
			10:00	1.07
			12:00	1.28

Table I shows that the clearly defined stratum found on the morning of the 6th was still at a depth of three meters at sunset, when the sun's rays are less intense than in the morning. After sunset, however, the band was broken up, a majority of the colonies sinking from one to three meters. At sunrise on the following morning the colonies were in the upper three meters and as the day progressed they migrated toward the surface until at noon over ninety percent were in the upper meter. The data on this gradual rise vary somewhat from hour to hour, but these variations are, in my opinion, due to experimental errors and do not represent changes in the position of maximal distribution.

TABLE III

Table Showing Temperature Range for the Upper Seven Meters of the Lake

	Depth in Meters							
	0	1	2	3	4	5	6	7
Temp. in deg. Cent.....	25.0	24.4	24.0	23.1	22.3	21.4	20.5	19.2

It is well known that Volvox responds to various stimuli. Changes in temperature, chemical composition of the water, direction of the

force of gravity, or light may cause a change in the position of colonies. The following table shows the temperature variations for the upper seven meters of the lake on the morning of July 6. Thermotaxis is not a factor in the problem before us since the difference between the upper and lower range is comparatively slight, while the differences between day and night temperatures are negligible.

The variable factors of the chemical environment are the gases in solution in the water. Birge and Juday (1) have studied this problem of dissolved gases particularly on Lake Mendota, which flows into Lake Monona, and at that time the conditions in the two lakes differed but little. They find that the carbon dioxide occurs in two forms, the fixed ($CaCO_3$ or $MgCO_3$) which is not available for the photosynthetic activities of the plant, and the half-bound ($CaCO_3.H_2CO_3$ or $MgCO_3.H_2CO_3$) which is such a loose combination that it is available for the use of algae. When the free carbon dioxide of the water has been exhausted the algae draw upon this supply of half-bound carbon dioxide and this produces an alkalinity of the water. The degree of alkalinity in Table IV is measured by the number of cubic centimeters of carbon dioxide that would be required to convert the normal carbonates into bicarbonates and thus give the water a neutral reaction. This difference, or degree of alkalinity, is the measure of the amount of the half-bound carbonates that have been utilized by the algae. The data of Birge and Juday for July 10, 1906, may be taken as typical for midsummer conditions in Lake Mendota (Table IV). The table shows that the oxygen was practically constant

TABLE IV

Gases Dissolved in the Water of Lake Mendota (after Birge and Juday, 1, p. 158). The Data Are in the Form of Number of Cc. of Dissolved Gas per Liter

Depth in Meters	Temp.	Half-bound CO_2	Fixed CO_2	Oxygen
0	24.2	−6.6	35.9	7.0
5	20.1	−6.1	36.2	6.9
8	17.7	−2.0	36.4	4.0

in the upper five meters, the region from which Volvox was collected, while the extent to which the available supply of carbon dioxide had been utilized shows that there was comparatively little difference in the first five meters. There is then no especially localized density in the amount of photosynthetic material or oxygen to cause a localiza-

tion of the alga in any particular place in response to a chemotactic stimulus.

Geotaxis, however, plays an important part in the distribution of Volvox. Mast (5) finds that, when a horizontal beam of light is thrown across an aquarium, the colonies tend to move horizontally towards the source of light, but the force of gravity pulls them downwards so that they sink very rapidly until, as a result of the difference in weight between the anterior and posterior ends, they become vertically oriented and begin to swim upwards. He also finds that after an aquarium has been in darkness some four or five hours many colonies are at the surface of the water. This probably explains the condition noted at 9:20 P.M. After sunset gravity is the only stimulus operating upon the colonies. Since they are heavier than water they tended to settle, and sank from one to three meters below their position at 7:45 P.M. Some time between 9:20 and sunrise the next morning the colonies became vertically oriented and swam upwards, so that at sunrise they were in the upper three meters of the lake. The physiological condition of the colony affects this upward swimming. Mast (5) observed that the colonies would be lying motionless on the bottom of the aquarium after standing in darkness for some hours. When illuminated they did not respond immediately, but after a while slowly began to move in all directions, later becoming normally active and moving towards the light. He applied the term "dark rigor" to describe their condition when lying on the bottom. Apparently there was no "dark rigor" in the Volvox colonies in Lake Monona since they were found in the upper part of the lake at sunrise.

Light is by far the most important factor governing the distribution of Volvox. The response to light by Volvox has been noted by many naturalists, but Oltmanns (7), Holmes (2) and Mast (5, 6) are the only ones to have made quantitative studies. Oltmanns produces a graded intensity in the illumination of an aquarium by passing a beam of light through a hollow glass prism filled with a mixture of India ink and gelatine. The narrow end of the prism permits most of the light to pass through while the broad end of the prism absorbs the greater portion of it. He finds that the colonies collect at a given light intensity, but if the general illumination of the prism is lowered, as when a cloud passes over the sun, the colonies move toward a region of formerly greater illumination. The experimental errors in this method have been analyzed by Mast (6). Holmes's studies are largely

concerned with an attempt to explain the mechanics of orientation. He notes that Volvox is negatively phototactic in strong light, while in very weak light colonies exhibit no pronounced phototactic movement, lying quietly or rolling about sluggishly. The most extensive studies are those of Mast, whose "light grader" for producing a graded intensity of the illumination overcomes the experimental errors of Oltmanns' apparatus. His investigations center about the orientation of the colony with respect to the direction of the rays of light, and he concludes that direction of movement in a colony is determined by the difference in intensity of illumination of the two sides of the colony rather than by the colony placing itself in a specific position with respect to the rays of light.

The explanation of the formation of the stratum at a depth of three meters on July 6 seems to me to rest on a heliotactic basis. Several investigators have noted that Volvox is positively heliotactic in weak light and negatively heliotactic in strong light. The formation of this stratum was probably caused by the colonies moving into the region of optimum illumination in the same way that Oltmanns (7) found in his experiments. This condition remained fairly constant during the day. The fact that the stratum did not move upwards when the illumination decreased at sunset is, however, not in accordance with this view. The dropping of the colonies from the three-meter level after sunset has already been ascribed to geotaxis.

The condition found at sunrise on the following day has been explained by the tendency of colonies to swim upwards in darkness after the greater weight of the posterior end orients them into a vertical position. There is the possibility that heliotaxis had been operating for some time before the collection was made at 4:25 A.M., since there is considerable illumination before the sun actually appears. Three quarters of an hour before sunrise a man could not be distinguished at a distance of seventy-five yards. If, then, we ascribe this movement of the colonies to the surface to heliotaxis they must have traveled three meters or more during the half hour of faint illumination. It would be reasonable to assume that this rate of movement continued during the hours after sunrise; so, when we find that the colonies did not rise more than a meter between 4:30 and 6:30, it seems illogical to assume that the light before sunrise caused the change of position. This is especially true when we remember that Holmes (2) finds no pronounced phototactic movement of Volvox in weak light.

Table I shows that during the morning of the 7th there was a continued upward migration of the colonies until at noon practically all were in the upper meter. These results are wholly inconsistent with those of the previous day. The colonies were in the region of maximal sunlight where the amount of light was undoubtedly far in excess of the optimum. The measurements of the total illumination for the two days are practically the same, so that this movement cannot be accounted for on the basis of heliotaxis. It is a well-known fact that filamentous algae like *Spirogyra* rise and fall during the day because of a buoyancy from the adherent oxygen bubbles formed during photosynthesis. The suggestion that a buoyancy of this type may have carried the colonies to the surface is inadequate since the illumination of the two days was constant. The variation in the gases dissolved in the waters of Lake Mendota has been discussed above and the assumption made that present-day conditions are the same. During recent years the "sludge" from the city sewage disposal plant has been emptied into Lake Monona, and it is just possible that the oxygen requirements of the undecomposed sewage coupled with the lack of aeration in the upper five meters by wave action produced a deficiency of oxygen that caused the migration of the colonies into the region of intense illumination the second day.

The third day's investigations showed but few colonies. The length of the hose did not permit sampling below eight meters, but all depths to the bottom were sampled where the water was less than eight meters deep and few colonies were found where the previous day the shallow water had been a bright green. This disappearance of the alga overnight, while very remarkable, is not inexplicable. We know from the studies of Marshall Ward (3) and others that direct sunlight kills many algae. Mast (5) has shown that this is also true for Volvox. Since the colonies were in full sunlight on the 7th, it is not at all improbable that they were killed by this strong light and gradually sank to the bottom. We should therefore find no colonies on the 8th or on succeeding days.

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A COMPARISON OF SALT REQUIREMENTS FOR YOUNG AND FOR MATURE BUCKWHEAT PLANTS IN WATER CULTURES AND SAND CULTURES

JOHN W. SHIVE AND WILLIAM H. MARTIN

In recent experimental work[1] bearing on the growth of plants in nutrient solutions, it has been demonstrated that the three salts, mono-potassium phosphate, calcium nitrate, and magnesium sulphate, in the proper proportions, in solutions of suitable concentrations, furnish a medium very well adapted to general culture work. This three-salt solution produces excellent growth and has the additional advantage of being the simplest nutrient solution (with respect to the number of salts employed) which can be devised and still contain all the elements, except iron, essential for plant growth. To determine approximately the optimum salt proportions for wheat (*Triticum vulgare*) during the first four weeks after germination, and for buckwheat (*Fagopyrum esculentum* Moench) during the early period of growth from germination to flowering, an optimal series of 36 different solutions was employed. All the solutions had approximately the same total osmotic concentration value of 1.75 atmospheres, and the salts were so distributed as to include all possible sets of salt proportions, when the partial concentrations of the three components were made to vary by increments equal to one tenth of the total concentration. Each solution contained the usual trace of iron as ferric phosphate, in addition to the three salts. Tests of the 36 different proportions of the nutrient salts showed that the best growth of wheat tops was produced by a solution containing the three salts in the following volume-molecular proportions: KH_2PO_4, 0.0180 m.; $Ca(NO_3)_2$, 0.0052 m.; and $MgSO_4$, 0.0150 m. The best growth of buckwheat tops and of roots was obtained in a solution in which the partial volume-molecular concentrations of the three salts were: KH_2PO_4, 0.0144 m.; $Ca(NO_3)_2$, 0.0052 m.; and $MgSO_4$, 0.0200 m.

These proportions of the three salts are, of course, to be considered

[1] Shive, J. W. A three-salt nutrient solution for plants. Amer. Journ. Bot. 4: 157–160. 1915.

approximately optimum only for wheat and buckwheat during the first four weeks of their growth after germination. It seemed highly desirable to determine whether the salt proportions required for optimum growth during the later periods of development, for the maturing of the plants, and for seed production, are the same or different from those above given for approximately optimum growth of seedlings. An attempt was made in this direction, and it is the purpose of the present paper to state the main results of the work in a preliminary way. The tests were carried out with buckwheat, first in water cultures. Similar tests were then made with sand cultures.

The methods employed with water cultures were the same as those previously employed by Shive[2] in his work with wheat. The seeds were germinated and the seedlings were mounted (three seedlings to each culture) in the manner described in the publication just cited (pp. 343–344), and were then placed in the culture vessels, which consisted of pint "Mason" jars. Each culture vessel contained 515 cc. of the same solution. This solution had the salt proportions, above given, which produced the highest yield of buckwheat tops during the first four weeks of growth after germination. All the seedlings were grown in this solution (with renewal of solutions every five or six days) during the first 24-day period. At the end of this time the plants were nearly alike. Some of the plants of each culture were in bloom and all the plants appeared healthy and vigorous. At the end of this early growth period the cultures were transferred to the 36 different solutions of the optimal three-salt series, each with a total osmotic concentration value of 1.75 atmospheres. These included all the possible sets of salt proportions for increments of change equal to one tenth of the total osmotic concentration. One culture was also transferred to Knop's solution and one to Tottingham's[3] best solution for wheat, each with a total concentration equal to that of the three-salt solutions. The cultures were now continued, with renewal of solutions as before, until the seeds were mature. This required a time period of 28 days. The first series was conducted from October 21 to December 9. This was repeated between December 9 and January 20.

[2] Shive, J. W. A study of physiological balance in nutrient media. Physiol. Res. 1: 327–397. 1915.

[3] Tottingham, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. 1: 133–345. 1914.

The solution giving the best growth of buckwheat tops contained the three salts in the following volume-molecular partial concentrations: KH_2PO_4, 0.0108 m.; $Ca(NO_3)_2$, 0.0130 m.; and $MgSO_4$, 0.0100 m. This solution also produced the highest yield of roots. It will be observed that these proportions are not at all the same as are those which produced the maximum yield of buckwheat tops and of roots during the early period of growth, from germination to flowering. It thus appears that the salt proportions of the best physiologically balanced solutions for buckwheat during the early period of growth are totally different from those which characterize the best physiological balance for these plants during the later period, from flowering to maturity.

The optimal series of solutions with a total osmotic concentration value of 1.75 atmospheres was repeated in sand cultures, with renewal of solutions as in the tests with solution cultures. The renewal of solutions in sand cultures was accomplished by a method similar to that employed by McCall.[4] The containers, consisting of earthenware pots glazed inside and outside, each held 2,500 g. of washed, air-dry, white quartz sand. The sand in each pot was flooded with an initial application of 750 cc. of solution, the excess of which was then withdrawn, leaving the sand with a moisture content of approximately 15 percent of the weight of the air-dry sand The excess solution was withdrawn through a glass tube extending to the bottom of the pot, instead of through a metal tube sealed to the bottom of the pot, as was done by McCall. This method was found entirely satisfactory and prevented the possibility of any metal coming in contact with the solution.

The first series of sand cultures was continued, with renewal of solutions (250 cc. of solution with each renewal) every three or four days, until the plants were in bloom. This required a time period of 25 days after the seedlings were transferred to the sand cultures. The series was then repeated. These two series of sand cultures, one conducted in May and the other in June, corresponded to the two series of solution cultures conducted during the early period of growth, from germination to flowering.

The solution in sand culture giving the highest yield of buckwheat tops during this early period of growth contained the three salts in the

[4] McCall, A. G. A new method for the study of plant nutrients in sand cultures. Amer. Soc. Agron. 7: 249–252. 1915.

same volume-molecular partial concentrations as did the solution giving the highest dry weight of tops and of roots in the corresponding series of water cultures. In the solution of the sand culture which gave the highest dry weight of roots[5] the volume-molecular partial concentrations were not the same as were those in the solution giving the highest dry weight of tops. The volume-molecular proportions of the three salts which gave the highest yield of roots were: KH_2PO_4, 0.0180 m.; $Ca(NO_3)_2$, 0.0104 m.; $MgSO_4$, 0.0050 m.

To determine the salt proportions required to produce approximately optimum growth of buckwheat in sand cultures during the period between flowering and maturity, the following procedure was adopted: The plants were first grown to the flowering stage in sand cultures, all of which contained the same solution. This solution had the salt proportions giving the best growth of tops in sand culture and in water culture during the early period of growth, from germination to flowering. The solutions were renewed every three or four days. At the end of this early 25-day period the cultures were all nearly alike, the plants throughout showing exceptional uniformity.

The solutions in the sand cultures were now replaced by the 36 different solutions of the optimal three-salt series. This was accomplished by passing through the sand of each culture (after first adding sufficient distilled water to bring the entire system back to its original weight) a triple portion (750 cc.) of the new solution, thus flushing out the old solution and replacing it with the new. At the same time the solution of one culture was replaced with Knop's solution, and that of another with Tottingham's best solution for wheat, each with a total osmotic concentration value of 1.75 atmospheres.

The series was now conducted, with renewal of solutions as before, until the seeds were mature. This second growth period extended over a time interval of 30 days. The series was then repeated. The first of these two series was conducted from April 25 to June 19. The second series, which was just like the first, was carried out between July 2 and August 27.

The highest yield of tops, of roots, and of seeds, was obtained from the same culture. The solution of this culture contained the three salts in the same volume-molecular partial concentrations as did the

[5] The dry weights of roots from the sand cultures were obtained by a method similar to that employed by McCall, Physiological balance of nutrient solutions for plants in sand cultures. Soil Science 2: 207–253. 1916.

solution of the corresponding series of water cultures which produced the highest dry weights of tops and of roots. These volume-molecular partial concentrations are: KH_2PO_4, 0.0108 m.; $Ca(NO_3)_2$, 0.0130 m.; and $MgSO_4$, 0.0100 m. This set of salt proportions is not at all the same as that which produced the highest yields of tops or of roots in sand cultures during the early growth period, from germination to flowering.

The results here presented are brought together in the following table which gives also the dry weight yields of tops, roots, and seeds, expressed in terms of the corresponding yields from Knop's solution and from Tottingham's best solution for wheat tops, taken as unity. Each of these data represents the averages from two corresponding series.

Relative Dry Weights, and Salt Proportions Producing Maximum Yields of Buckwheat with an Optimal Series of Three-salt Solutions, in Water Cultures and in Sand Cultures, during Two Different Physiological Growth Periods

			Volume-molecular Partial Concentrations			Yields of Tops, Roots, Seeds, Relative to Those from	
			KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Knop's Solution taken as 1.00	Tottingham's Solution taken as 1.00
Buckwheat grown to flowering (early growth period)	Water cultures	Tops	0.0144	0.0052	0.0200	1.61	1.33
		Roots	0.0144	0.0052	0.0200	1.58	1.27
	Sand cultures	Tops	0.0144	0.0052	0.0200	1.38	1.25
		Roots	0.0180	0.0104	0.0050	1.47	1.16
Buckwheat grown to maturity (late growth period)	Water cultures	Tops	0.0108	0.0130	0.0100	1.40	1.35
		Roots	0.0108	0.0130	0.0100	1.50	1.39
		Seeds	0.0108	0.0078	0.0200	1.28	1.74
	Sand cultures	Tops	0.0108	0.0130	0.0100	1.40	1.26
		Roots	0.0108	0.0130	0.0100	1.27	1.26
		Seeds	0.0108	0.0130	0.0100	1.24	1.17

From these results it is at once clear that the salt proportions required to produce the best physiological balance for buckwheat during the two different growth periods here considered differ markedly and in the same manner with water cultures and with sand cultures. It appears that the best physiological balance of salt proportions for buckwheat with this optimal three-salt series of solutions is not greatly disturbed when these solutions, with frequent renewals, are employed in sand cultures. This is clearly shown by the fact that the salt pro-

portions producing maximum yields of tops in water cultures are in perfect agreement with those giving the highest dry weights of tops in the corresponding series of sand cultures, during each of the two different growth periods. This is further emphasized by a similar agreement between the salt proportions producing maximum yields of roots in the series of water cultures and in the corresponding series of sand cultures, during the late growth period, from flowering to maturity, although there is no such agreement between the salt proportions producing the maximum yield of roots in water culture and those giving the highest dry weight of roots in sand culture, during the early growth period. The salt proportions which produced the highest yield of seeds in water culture are not like those which gave the highest dry weight in sand culture. In this connection it should be mentioned, however, that the water cultures were conducted at a season of the year when insects were absent, and pollination by artificial means was perhaps imperfectly accomplished. The plants of the sand cultures, on the other hand, were in bloom at a time when insects were abundant, and these had free access to the plants. It will be observed that in this series the salt proportions giving the best growth of tops and of roots are the same as those which produced the highest yield of seeds.

It is interesting to observe that with three out of the four average series here represented, there is a definite correlation between the growth of tops and that of roots, as is clearly indicated by the perfect agreement between the salt proportions producing the highest dry weight of tops and those giving the maximum yields of roots.

As the relative dry weight values in the last two columns of the above table indicate, the three-salt mixture with a total osmotic concentration value of 1.75 atmospheres, and with proper salt proportions, produced markedly higher yields than did either Knop's or Tottingham's solution with the same total osmotic concentration, for the two different physiological growth periods here considered.

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CELL MEASUREMENT AS AN AID IN THE ANALYSIS OF QUANTITATIVE VARIATION*

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The inheritance of quantitative characters is now commonly interpreted on a basis of multiple Mendelian factors. It is not too much to say, however, that relatively little has been done to identify these factors. Enough of them have ordinarily been postulated to account mathematically for the results in any given case, but a biological analysis has seldom been attempted. One must except the work of Emerson,[1] who has found that the height of a bean plant is due to at least three, and probably more, independent factors, namely, the number of internodes, the length of the individual internodes, and the habit of growth, whether determinate or indeterminate. It has seemed desirable to us to proceed still further with the analysis and we have taken up first the problem of internode length.

Variation in the length of an internode may be correlated either with variation in the number or size of the constituent cells. *A priori*, therefore, there are at least two factors whose relative importance it should be possible to measure in any given case. If, in addition, it should be possible to demonstrate the hereditary behavior of either factor, or of both, and to determine the fluctuations of both due to environment, the whole problem of the inheritance of quantitative characters would become much more concrete and would be brought correspondingly nearer to a solution.

Before proceeding to the analysis of genetic variations, we undertook to test the proposed method by applying it to a variation brought about by environmental agencies. The etiolated epicotyl of *Phaseolus multiflorus* Willd. was chosen, because it is much longer than the normal epicotyl grown in light. Not only do the results demonstrate the feasibility of resolving internode length into less complex characters, but they are also of considerable interest *per se*, adding, as they do, to

* Papers from the Department of Botany of the University of Michigan, no. 161.

[1] Emerson, R. A. A genetic study of plant height in Phaseolus vulgaris. Nebr. Agr. Exp. Sta. Res. Bull. 7: 1–73. 1916.

the none too ample quantitative data concerning the effect of light on the growth and division of cells.

The classical work on etiolation is that of Gregor Kraus,[2] who made a large number of cell measurements in both normal and etiolated stems. He concluded that the greater length of etiolated internodes was due almost entirely to the greater length of the cells, but that a certain part of the increase over the normal internodes had to be ascribed to an increase in the number of cells. Kraus made thousands of measurements, and, as a whole, his work was painstakingly done and of substantial value. He did not, however, make enough measurements in individual cases to establish true means, or to determine ranges of variation, and he likewise failed to distinguish, in measurements of epidermis, between primary and secondary cells. Moreover, as we shall show further on, his work must share with that of others the criticism that it was probably based upon material that was not strictly comparable. The comparability of individual plants grown under different conditions can only be assured by determining the range of fluctuating variation of a sufficient number of plants grown under each condition.

The entire subject of etiolation was reviewed in 1903 by MacDougal,[3] to whose memoir the interested reader should turn for references to the extensive literature. With regard to the epidermal cells of etiolated stems his conclusion (*l. c.* p. 247) is as follows: "Epidermal cells were found to be as long as the normal in all instances, except in *Menispermum* (*canadense*), in which species alone the superficial measurements were less than in normal stems. The epidermal cells showed an increase in all dimensions in a great number of instances in which a multiplication of these elements had also ensued. Among the earlier investigators various contentions arose as to whether the excessive elongation of stems was accompanied by increase in size, or by increase in number, of the epidermal cells, the conclusions of the various workers being based upon the small number of species examined. It is to be seen, however, that no general law has been discovered by which the action of the epidermis in darkness may be predicated." Except for the explicit case of Menispermum, Mac-

[2] Kraus, Gregor. Ueber die Ursachen der Formänderungen etiolierender Pflanzen. Jahrb. Wiss. Bot. 7: 209–260. 1869–'70.

[3] MacDougal, D. T. The influence of light and darkness upon growth and development. Mem. N. Y. Bot. Gard. 2: 1–319. 1903.

Dougal's work (he gives few measurements, but the figures are drawn to scale) seems to bear out Kraus's conclusion that the elongation of an etiolated stem is due to increase in both number and size of cells. Kraus's data for Phaseolus, however, were in conflict with his own general conclusion. He found that etiolated internodes of *Phaseolus vulgaris* L., although elongated, had fewer cells than normal internodes, seemingly indicating that the entire increase in length was due to cell size. Our work leads us to believe that in this case there was an unusually extreme error due to failure to make cell measurements from plants of comparable position in the range of fluctuating variation.

In our experiments seeds of the scarlet runner bean (*Phaseolus multiflorus* Willd.) were grown in complete darkness and in light. The length of the epicotyls of 80 etiolated and 92 normal plants was measured; the symmetrical frequency distributions gave the following constants:

	Range of Variation	M	σ	CV
Grown in darkness	168–517 mm.	305	71.4	23.4
Grown in light	30–141 mm.	85	16.9	19.9

Thus, the etiolated stems were 3.6 times as long as those grown in the light, and had only a slightly greater coefficient of variation.

An extremely long normal epicotyl (141 mm.) was taken for cell measurements. For our purposes it was more favorable than one of modal length, in that the epidermis was surely made up of almost the maximum number of cells for a normal epicotyl. A rough check on this statement is afforded by multiplying the length of the normal epicotyl (141 mm.) by the factor 3.6, the ratio between the mean length of normal and etiolated stems. The result is 508 mm., in sufficiently close agreement with the actual maximum length (517 mm.) for an epicotyl grown in darkness. If, therefore, an etiolated epicotyl of less extreme position in the variation curve should contain more cells, it would prove conclusively that the effect of light is to retard cell division as well as to diminish cell size, and that in Phaseolus, as in other plants, the etiolated internodes have more cells than the normal. The etiolated epicotyl chosen for cell measurements was 372 mm. long; *i. e.*, it was exceeded in length by 20 percent of the variates, and corresponded to a normal epicotyl 133 mm. long.

Each epicotyl was divided into 10 equal segments, and in each segment 100 primary epidermal cells, taken at random, were measured. Thus for each epicotyl 1,000 primary cells were measured, uniformly distributed along its length. Many of these primary cells were divided by transverse walls into secondary cells, which were also measured, bringing the number of measurements up to 2 or 3 thousand for each epicotyl. Kraus had observed in his work that the ends of many epidermal cells were not even approximately perpendicular to the longitudinal axis, but were pointed. He wrote: "It is a common phenomenon that the cells, at any rate those of the epidermis, do not remain parenchymatous during elongation in etiolation, but like the elongating wood-forming cambial cells, become completely prosenchymatous, the long, sharp ends dove-tailing in between one another." This condition occurs in the normal as well as in the etiolated epidermis, but a further fact is that many cells do have perpendicular end walls, and appear, as we have already indicated, to have been formed at a late stage in the development of the internode. With regard to the latter point, MacDougal says of epidermal cells of etiolated stems in general: "The epidermis, in common with many other tissues, does not advance beyond a certain primary stage of development, and retains the power of growth and division in the cells during a much longer period than in the normal plant; consequently it can respond to stresses and other factors, which may cause it to undergo increase in size, alterations in form, or multiplication of the cells by division." In the epidermis of *Phaseolus multiflorus* it proved to be a simple matter to distinguish by the character of the end walls between the primary cells, formed by division of the primary meristem, and secondary cells, formed by subsequent divisions. The prosenchymatous shape of the former is obvious, even after they have divided into secondary cells. The latter have at least one transverse end wall, as in any typical parenchymatous cell. (See figs. 1 and 2, reduced to scale from camera lucida drawings.) Measurements were made of cells taken at random, but were so recorded so as to distinguish undivided primary from secondary cells. All the secondary cells derived from a single primary were measured, and their lengths added to obtain the total length of the epidermis derived from it.

It is obvious that in any discussion of cell length as a factor in internode length, one must distinguish carefully between primary and secondary cells. Our tables give statistical data (1) for primary

cells (undivided, divided, and taken at random), (2) for cells taken at random, whether undivided primary cells or secondary cells, and (3)

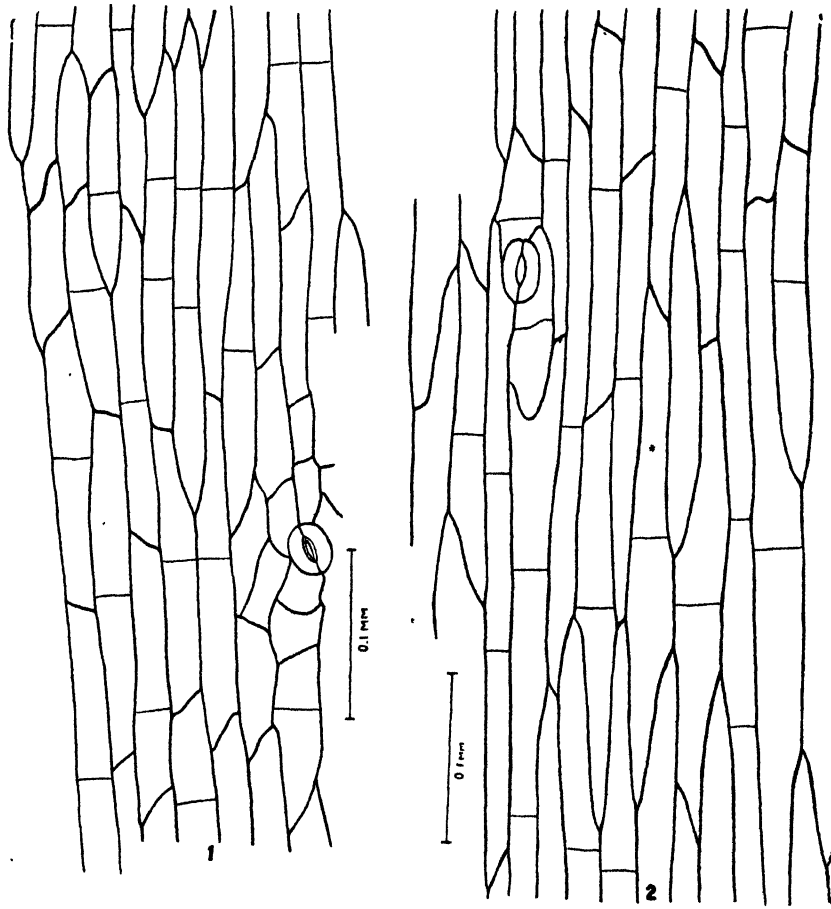


FIG. 1. Epidermis from epicotyl of *Phaseolus multiflorus*, grown in the light. (Material chosen from a section in which the primary cells were of the same mean length as those of the whole internode.)

FIG. 2. Epidermis from epicotyl of *Phaseolus multiflorus*, grown in the dark. (Material chosen from a section in which the primary cells were of the same mean length as those of the whole internode.)

for secondary cells alone. The measurements of undivided primaries proved to be remarkably interesting, for they tend to indicate that in

Phaseolus there is a physiological limitation to the length which can be attained by a cell without undergoing division.

Plant physiologists recognize, of course, that there is presumably for each kind of cell a specific size at which division takes place. The evidence, in the case of primary meristem, is based upon such experiments as those of Newcombe[1] in which growing tips were incased in gypsum, in order to prevent growth by mechanical means. Under such conditions the primary meristem ceases division, and does not resume it until the release of the pressure permits the growth of the cells to the specific size at which division takes place. The somewhat differentiated epidermal cells present a different condition. The cells are in a state of extension, accompanied by increase in volume of the vacuole, but not, as far as known, by any increase in the amount of protoplasm. Although it is quite in accord with expectation to find that cells in course of extension, providing they retain the meristematic function, should have a specific size for division, it is nevertheless a distinct gain to have additional data bearing upon the subject.

TABLE I

Length in Mm. of Primary Epidermal Cells (including both Divided and Undivided Cells) of Epicotyl of Phaseolus multiflorus Grown in Light. (The Sections are Numbered from the Basal Tenth (No. 1) upwards)

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.030–.057	9	12	7	1	0	1	1	0	0	2	33
.060–.087	47	45	37	13	2	11	3	6	5	9	178
.090–.117	37	25	33	24	18	15	13	29	15	21	230
.120–.147	7	17	22	26	23	26	24	20	33	26	224
.150–.177		1	1	21	22	20	27	17	23	23	155
.180–.207				12	12	15	18	10	15	15	97
.210–.237				2	12	6	8	11	4	3	46
.240–.267				1	5	5	4	1	3	1	20
.270–.297					2	1	2	2	1		8
.300–.327					3			3	0		6
.330–.357					1			0	1		2
.360–.387								1			1

Tables I and VI give the frequency distributions for the primary cells, disregarding secondary divisions. That is to say, the measurements were taken from end to end of the sharp-pointed outline of the

[1] Newcombe, F. C. The influence of mechanical resistance on the development and life-period of cells. Bot. Gaz. 19: 149–157, 191–199, 229–236. 1894.

TABLE II

Length in Mm. of Undivided Primary Cells of Epicotyl of Plant Grown in the Light

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.030–.057.....	9	12	7	1	0	1	1	0	0	2	33
.060–.087.....	47	45	37	13	2	11	3	6	5	9	178
.090–.117.....	37	25	31	22	17	14	9	28	13	18	214
.120–.147.....	7	17	21	19	12	11	11	6	14	5	123
.150–.177.....		1	1	10	6	4	4	0	5		31
.180–.207.....				1		2		0			3
.210–.237.....								1			1

TABLE III

Lengths in Mm. of Divided Primary Epidermal Cells of Epicotyl of Plant Grown in Light

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.090–.117.....			2	2	1	1	4	1	2	3	16
.120–.147.....			1	7	11	15	13	14	19	21	101
.150–.177				11	16	16	23	17	18	23	124
.180–.207.....				11	12	13	18	10	15	15	94
.210–.237.....				2	12	6	8	10	4	3	45
.240–.267.....				1	5	5	4	1	3	1	20
.270–.297.....					2	1	2	2	1		8
.300–.327.....					3			3	0		6
.330–.357.....					1			0	1		2
.360–.387.....								1			1

TABLE IV

Length in Mm. of Epidermal Cells Taken at Random, Including Undivided Primary Cells and Secondary Cells, from Epicotyl of Plant Grown in Light

Class	Section										Entire Internode
	1*	2*	3	4	5	6	7	8	9	10	
.003–.027.....	0	0	0	0	0	0	2	0	0	0	2
.030–.057.....	9	12	10	8	9	7	10	9	10	10	94
.060–.087.....	47	45	36	36	38	40	42	40	49	48	421
.090–.117.....	37	25	32	30	33	32	35	40	27	37	328
.120–.147.....	7	17	21	15	14	16	10	11	12	5	128
.150–.177.....		1	1	10	6	3	1		2		24
.180–.207.....				1		2					3

* These sections contained no divided primary cells.

group of cells derived from the original primary cell, in case the latter had undergone division. In many cases, of course, the shorter primary cells were undivided. Some mistakes in the differentiation of primary from secondary cells were doubtless made, but not enough to vitiate

TABLE V

Length in Mm. of Secondary Cells of Epicotyl of Plant Grown in the Light

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.003–.027	0	0	0	0	0	0	2	0	0	0	2
.030–.057			3	8	10	9	9	15	11	12	77
.060–.087			3	30	41	47	52	44	57	56	360
.090–.117				25	35	35	30	33	25	31	214
.120–.147				4	11	9	7	8	7	1	47
.150–.177				1	3						4

the results. In Tables II, III, VII and VIII the divided and undivided primary cells are separately enumerated. In Tables IV and IX are given the measurements of undivided primary and secondary cells, taken at random. The data of these two tables are therefore comparable with those of Kraus (l. c.). Tables V and X concern the secondary cells only. Tables XI and XII give a convenient summary of the data in Tables I to X.

TABLE VI

Length in Mm. of Primary Epidermal Cells of Epicotyl of Plant Grown in Dark. The Cells were Taken at Random, without Regard to Whether or Not they had Undergone Secondary Division

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.060–.117	8	1	5	4	1	1	4	4	7	12	47
.120–.177	42	9	16	15	8	14	17	11	13	23	168
.180–.237	34	24	22	28	41	21	21	19	16	22	248
.240–.297	11	30	30	30	18	27	29	28	26	14	243
.300–.357	5	23	16	11	18	15	17	12	18	11	146
.360–.417		8	8	7	7	13	6	8	12	9	78
.420–.477		4	2	1	5	8	4	8	6	7	45
.480–.537		1	1	2	2	1	2	8	2	2	21
.540–.597				2				2			4

Beginning with section 1, and reading downward in all the columns in Table XI, it is seen that under both conditions of growth the epidermal cells, whether primary, secondary, or taken at random, show a

gradual increase in length from the base upward, to a maximum. After the maximum mean length is reached, there is a decrease toward the upper end of the internode, but not a decrease to the minimum which occurs at the base. The gradual increase in cell size may possibly be partially accounted for by increase in the amount of water available during the elongation of the internode. When the seedling is very young the root system is simple, and relatively inadequate. Moreover, the differentiation of conductive tissue is not complete. Consequently the cells cease expanding earlier than do subsequently formed cells, which have the advantage of a copious water supply and a more highly developed conductive system. In attributing to water supply a possible influence upon the size of epidermal cells, we do not commit ourselves to any theory as to the cause of extension. Whether extension is due to turgor pressure or not, the process could not take place at all without sufficient water to keep the protoplasm in contact with the cell wall, and that amount would hardly be measurably different for a moderately rigid cell wall whether the turgor pressure were great or small.

TABLE VII

Length in Mm. of Undivided Primary Epidermal Cells of Epicotyl of Plant Grown in Dark

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.060–.117	7	1	2	4	1	1	4	3	6	11	40
.120–.177	30	3	4	10	3	9	12	6	4	19	100
.180–.237	4			3	1	4	6	4	2	6	30
.240–.297							1	3		2	6

TABLE VIII

Length in Mm. of Divided Primary Epidermal Cells of Epicotyl of Plant Grown in Dark

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.060–.117	1	0	3	0	0	0	0	1	1	1	7
.120–.177	12	6	12	5	5	5	5	5	9	4	68
.180–.237	30	24	22	25	40	17	15	15	14	16	218
.240–.297	11	30	30	30	18	27	28	25	26	12	237
.300–.357	5	23	16	11	18	15	17	12	18	11	146
.360–.417		8	8	7	7	13	6	8	12	9	78
.420–.477		4	2	1	5	8	4	8	6	7	45
.480–.537		1	1	2	2	1	2	8	2	2	21
.540–.597				2				2			4

Whatever the cause of the variation within the internode, it is obvious that tissues for cell measurement should be taken from strictly comparable regions in the plant. It would seem to be a safe rule that in studies of the stem the tissue should come from the middle of the internode, unless a sufficiently large number of cells are measured so that an equal number can be taken from each aliquot part of the internode.

TABLE IX

Length in Mm. of Epidermal Cells Taken at Random Including Undivided Primary Cells and Secondary Cells from Epicotyl of Plant Grown in the Dark

Class		Section									Entire Internode
		2	3	4	5	6	7	8	9	10	
.030–.057	2	0	3	0	1	0	0	2	3	3	14
.060–.087	22	22	24	8	8	11	5	6	12	13	131
.090–.117	41	32	37	25	48	31	21	23	19	36	313
.120–.147	21	30	27	37	29	33	39	25	33	17	219
.150–.177	12	13	9	25	10	17	22	16	15	22	161
.180–.207	1	3		5	4	7	9	5	13	8	55
.210–.237	1					1	3	11	3	1	30
.240–.267							1	7	2		10
.270–.297								3			3
.300–.327								2			2

TABLE X

Length in Mm. of Secondary Epidermal Cells of Epicotyl of Plant Grown in the Dark

Class	Section										Entire Internode
	1	2	3	4	5	6	7	8	9	10	
.030–.057	2	3	3	9	1	0	0	2	3	2	25
.060–.087	31	24	25	32	8	11	6	5	10	10	162
.090–.117	44	37	37	42	49	32	23	22	16	33	325
.120–.147	18	27	26	21	29	30	42	24	33	18	268
.150–.177	5	9	9	4	10	17	18	20	18	25	135
.180–.207				2	3	7	8	4	14	8	46
.210–.237						3	3	11	4	2	23
.240–.267							1	6	2	2	11
.270–.297								4			4
.300–.327								2			2

Kraus assumed that the quotient of the length of the internode divided by the mean length of the cells would bear a simple relationship to the number of cells making up the length of the internode. Since there is considerable dovetailing of the cells between one another, the quotient does not, of course, indicate the number of cells in a

TABLE XI

Mean Length in Mm. of Epidermal Cells of Epicotyl of Phaseolus multiflorus, based upon Data Given in Tables I–X

Section	Plant Grown in Light					Plant Grown in Darkness				
	All Primary Cells	Undivided Primary Cells	Divided Primary Cells	Undivided Primary and Secondary Cells	Secondary Cells	All Primary Cells	Undivided Primary Cells	Divided Primary Cells	Undivided Primary and Secondary Cells	Secondary Cells
1	.087	.087	(None)	.074	(None)	.187	.175	.216	.109	.102
2	.090	.090	(None)	.087	(None)	.273	.135	.274	.114	.109
3	.096	.096	.090	.092	.058	.250	.147	.258	.106	.108
4	.135	.115	.163	.097	.087	.225	.146	.276	.130	.100
5	.169	.122	.190	.096	.088	.267	.150	.250	.117	.119
6	.145	.112	.179	.094	.088	.280	.163	.292	.126	.130
7	.162	.124	.178	.085	.084	.259	.199	.288	.138	.135
8	.155	.093	.189	.087	.085	.291	.176	.316	.153	.158
9	.153	.117	.170	.086	.083	.264	.129	.283	.135	.141
10	.144	.097	.163	.083	.081	.242	.148	.300	.153	.163
Entire internode	.135	.102	.189	.085	.090	.258	.149	.282	.126	.124

TABLE XII

Statistical Constants Based upon Data Given in Tables I–X

Constants	Plant Grown in Light					Plant Grown in Dark				
	Primary Cells	Undivided Primary Cells	Divided Primary Cells	Undivided Primary and Secondary Cells	Secondary Cells	Primary Cells	Undivided Primary Cells	Divided Primary Cells	Undivided Primary Cells and Secondary Cells	Secondary Cells
M (mm.)	.135	.102	.189	.085	.090	.258	.149	.282	.128	.124
σ (mm.)	.053	.033	.040	.033	.026	.097	.025	.083	.040	.043
C. V.	39.2	32.4	26.0	38.8	28.9	37.6	16.9	29.4	31.2	34.7

vertical series, from node to node, but for the comparison of very similar material, such as ours, it probably affords quite as useful a measure of the cell number factor. It will be remembered that the normal internode chosen for cell measurements came at the extreme upper limit of the range of variation, and that the etiolated internode corresponded to a much shorter normal one, the two lengths being in the ratio 141 to 103. (The value 103 is obtained by dividing the actual length of the etiolated internode, 372 mm., by the factor 3.6,

thus correcting for the total effect of light.) As already explained, the choice of an extremely long normal internode obviated any possibility of failure to detect the cell number factor in the elongation due to etiolation, in case any such factor existed. The available evidence indicates that under relatively constant environmental conditions, variation in internode length is correlated with the number rather than with the size of cells. (*Teste* Kraus; results with long and short internodes of Philadelphus.) If it had been grown under normal conditions, therefore, the etiolated internode from which our measurements were made might have been expected to produce 27 percent fewer cells than the normal one with which it was compared, yet in the dark it actually produced 38 percent more, if we base the comparison upon primary cells, including both undivided and divided, or 78 percent more, if we base the comparison upon undivided primary and secondary cells, taken at random. There can remain no doubt, therefore, that the effect of light, directly or indirectly, is to retard cell division.

If a correction is made for the difference in the position of the two plants in the range of variation, the number of primary meristematic divisions in darkness shows an increase of 88 percent over the number in the light, accounting for 34 percent of the total increase in length, leaving 66 percent to be accounted for by increased extension of the cell or group of cells derived from each division. In case primary and secondary cells are not distinguished, it appears that the number of cells in etiolated internodes is greater by 142 percent than in the normal ones, and that 55 percent of the increase in length is due to the cell number factor, and only 45 percent to the cell size factor. Since Kraus's conclusions were based upon cells taken at random, as in the latter case, the discrepancy between his results for *Phaseolus vulgaris* and ours for *P. multiflorus* requires a further word of explanation. He found the entire increase in length to be due to the cell size factor, but that his material was not strictly comparable is indicated by the fact that he did not determine the fluctuating variation of the plants which he used. Consequently he could neither select comparable internodes in the first place, nor correct for their deviation from comparability. In our work we have determined the range of variation for both normal and etiolated seedlings, and have assumed that, within the limit of experimental error, the cell number factor and the cell size factor have the same relative weight in bringing about the elonga-

tion of all etiolated internodes grown under like conditions. Although it is unlikely that this assumption is wrong, it requires proof; we have not yet undertaken the labor of making enough measurements to place it beyond criticism. It must be admitted, however, that relative position in the frequency distribution affords a logical basis for the determination of comparability.

In order to establish a relationship between length of the primary cells and their division into secondary cells, the divided and undivided primary cells were separately enumerated, with the striking results shown in Tables II, III, VII, and VIII. In both normal and etiolated internodes there is a high correlation between length and condition with regard to division, the shorter cells in each cases being the undivided ones, which are but little more than half as long as the divided ones. There is a pronounced tendency for the short cells at the base of the internode to remain undivided, and this is particularly so in the case of the markedly shorter cells of the normal internode.

In comparing the data, one is impelled to speculate as to whether light directly retards division of the primary cells, or acts indirectly by retarding the extension of the cells, so that relatively few of them attain the specific size for division. The latter supposition is the more simple. Bearing it in mind, we observe that in the plant grown in the light, 59 percent of the cells are undivided, 41 percent divided. In the dark 15 percent are undivided, 85 percent divided. From the frequency distributions in Tables I and VI we find that all below 59 percent of the illuminated cells or 15 per cent of the etiolated cells would include all below a length of about 0.140 mm. Some divided cells are shorter, and some undivided ones are longer, the two classes approximately balancing one another.

Turning to Tables II and VII, we find that the length 0.240 mm. is exceeded only by a trivial number of undivided cells, whether primary or secondary. Conversely (Tables III and VIII) we find that in only a trivial number of divided cells does the sum of the lengths of the derived secondary cells fall below 0.120 mm. The conclusion appears reasonably justified that the range of length within which division generally takes place is 0.120 to 0.240 mm., and that the greater part of the divisions occur at a length not far from 0.140 mm.

The expression "specific size," used without qualification, refers, of course, to specific volume, and since our measurements concern only one dimension, length, they are insufficient to determine a specific

size for division, in any strict sense. Observation, unsupported by measurements in the case of the present material, but borne out by studies of epidermal cells in the case of the genus Oenothera,[5] leads to the conclusion that cells which attain an excessive length are usually very slender, and vice versa. On this ground it is possible to explain the great range of variation in the length attained by different cells before division. It is quite correct, however, to speak of specific mean length for division, which is simply the mean of the lengths at which division takes place in a large number of cells, and this is the constant which we have determined with some approximation to accuracy as 0.140 mm. in the stem epidermis of *Phaseolus multiflorus*.

It appears that the specific mean length for division is the same in both light and darkness. The mean length of undivided primary cells is indeed greater in the etiolated internode, but this fact is readily explained. In the light only 76 out of 1,000 primary cells taken at random were both undivided and longer than 0.140 mm.: 583 were undivided. In the dark, however, there were only 176 undivided primaries in 1,000, and a relatively larger number of them, 103, exceeded the putative specific mean length, 0.140 mm. The extension of a considerable number of cells to somewhat beyond the specific mean length would be expected to bring about the division of most of the cells in the lower part of the range within which division takes place, and to leave only the cells which, because of slenderness or some other cause, come within the extreme upper part of the range. The small number of undivided primaries in the material grown in the dark suggests that most of those that remain must have passed the specific mean length. It is therefore not surprising that their mean length is 0.149 mm., whereas in every other instance the mean length of undivided cells, whether primaries or secondaries, was found to be well below 0.140 mm.

The simplest assumption with regard to the effect of light is that it retards extension of the cells, and that as an indirect result there are fewer secondary divisions, since relatively fewer primary cells enter the range of length within which division takes place. With regard to the cell divisions in the primary meristem, it is clear that more of them take place in the dark than in the light, but there is no evidence with regard to the cause.

[5] Tupper, W. W. and Bartlett, H. H. The relation of mutational characters to cell size. Genetics 3: 93–106. 1918.

SUMMARY

The mathematical formulation of the results of size inheritance according to the multiple factor hypothesis should be paralleled by a biological analysis, the object of which is the identification of the several factors concerned. In such a biological analysis, it will inevitably be found that quantitative variations may be correlated with variation in either the number or the size of the constituent cells of the organisms or organs involved. Still other variations involve both cell number and cell size.

In the investigation of quantitative variations of a hereditary nature, it seems likely that the study by the histological method of reactions to the environment and of the obscure reaction known as "vigor of heterozygosis" will afford a means of correcting for these disturbing factors.

In connection with genetical studies in Phaseolus, we have made some studies of fluctuating variation due to the effect of light, one of the most disturbing factors concerned in size inheritance. The results are of considerable interest in themselves, and may be summarized as follows:

1. In *Phaseolus multiflorus*, growth in darkness results in the elongation of the internodes to 3.6 times the length of normal internodes grown in the light.

2. This increase in length is accounted for to the extent of 34 percent by increase in the number of divisions taking place in the primary meristem, the remainder of the increase being due to increase in length of the cell or group of cells derived from each primary division.

3. It is possible to recognize the group of secondary cells formed by division of a primary cell during its extension, since the outline of the primary cell is pointed at the ends, whereas the subsequently formed cross walls are approximately perpendicular.

4. There appears to be a specific mean length for division of the primary epidermal cells, with a value of about 0.140 mm., which is independent of light or darkness.

5. In consequence of the fact that the length for division is attained in a larger number of primary cells in the etiolated than in the normal internode, it is necessary, in appraising the relative importance of the cell number and cell size factors, to discriminate carefully between primary and secondary epidermal cells.

RESPIRATION AND CATALASE ACTIVITY IN SWEET CORN

CHARLES O. APPLEMAN

In a former paper[1] it has been shown that there is an invariable parallelism between respiratory intensity of potato tubers and the catalase activity in the expressed juice. An increase or decrease of

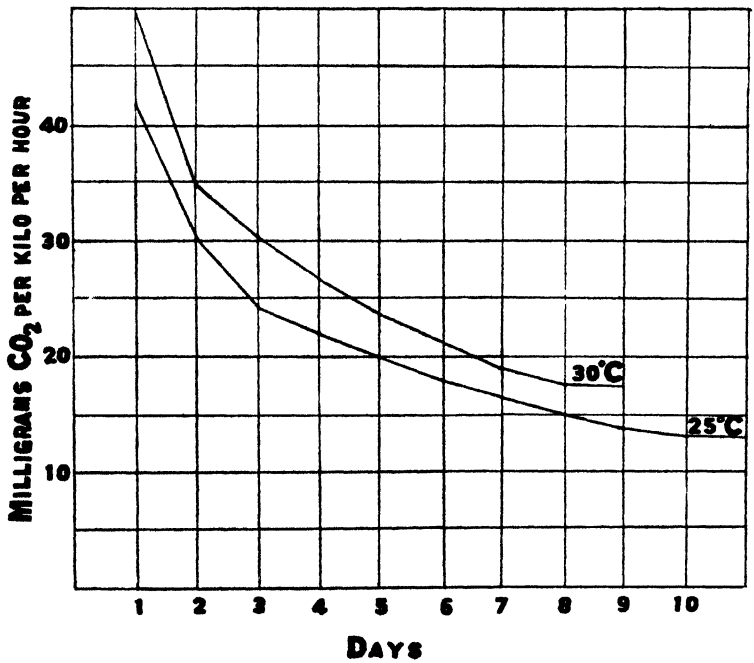


FIG. 1. Graphs showing respiratory intensity of sweet corn during storage.

respiration in the tubers is always accompanied by a corresponding increase or decrease of catalase in the juice. The experiments recorded

[1] Appleman, Chas. O. Relation of oxidases and catalase to respiration in plants. Amer. Journ. Bot. 5: 223–233. 1916.

in this paper show, in a very different kind of plant structure, a similar relation between respiration and catalase.

A very rapid change in respiratory intensity was found to occur in sweet corn in the milk stage, after it is pulled from the stalk. Respiration in the corn is very high when it is first pulled but falls off rapidly with storage, coming to a constant rate in eight days at 30° C. and in ten days at 25° C. At the end of five days' storage at 30° C. the respiratory intensity is about half of that found in the corn when first pulled.

The catalase activity in a collateral set of ears was measured immediately after the corn was pulled and after a period of storage, usually five days. The husks were not removed, and as a further precaution against loss of water the ears were wrapped in paraffined paper and stored in large moist chambers which allowed free access of air. The change in catalase activity during storage at 30° C. was determined by comparing measurements from the same ear. This was made possible by removing three rows of kernels for a catalase determination when the corn was first pulled and then removing three rows from the opposite side of the ear for a catalase measurement after a period of storage. The corn was ground to a pulp in a mortar and pressed in a small tincture press. About 0.5 gram of $CaCO_3$ was mixed with the corn to neutralize the acids liberated during grinding.

Of the milky extract 1 cc. was added to 25 cc. of distilled water.

After thorough mixing of the diluted extract, 2 cc. were allowed to act on 1 cc. of dioxygen, diluted in the proportion of 25 cc. of dioxygen to 75 cc. of water. Bunzell's simplified oxidase apparatus, graduated to read positive pressures, was employed for the catalase measurements as described in the former paper. The manometer readings were made at the end of 10 minutes' constant shaking at 30° C. Moisture determinations were made at the time of each catalase measurement. The percentage of moisture in the corn, under the conditions of storage, remained so nearly constant that it was unnecessary to correct for loss of water.

The catalase measurements recorded in Table I show a decline in catalase activity after five days' storage at 30° C. which is almost directly proportional to the decline in respiratory intensity in the corn after a like period of storage.

TABLE I

Catalase Activity in Sweet Corn

Ear	Manometer Readings Expressed in Centimeters of Mercury		
	When Pulled in Milk Stage	After Storage at 30° C.	
		2 Days	5 Days
1	4.0	3.0	2.0
2	4.3	3.6	—
3	4.2	3.6	—
4	3.8	—	1.9
5	3.7	—	1.2
6	3.8	—	2.5
7	4.2	—	2.6
Average	4.0		2.05

SUMMARY

Respiration in sweet corn in the milk stage is very high when the corn is first pulled. This high rate of respiratory activity falls off rapidly with storage. Catalase activity in a collateral set of ears showed a decline with storage which is almost directly proportional to the decline in respiratory intensity after a like period of storage.

The catalase activity of the expressed juice from both sweet corn and potato tubers is a fair index of the comparative intensity of respiration in the tissues.

The data from both plant and animal tissues available at present seem to justify the general induction that catalase action is invariably correlated with the oxidative processes involved in respiration.

LABORATORY OF PLANT PHYSIOLOGY,
MARYLAND AGRICULTURAL EXPERIMENT STATION

SOME NEW SPECIES OF INOCYBE

GEO. F. ATKINSON

Inocybe ammophila n. sp.

Gregaria, 4–6 cm. alta; pileo ochraceo vel brunneo, 2–7 cm. lato, convexo-expanso, dein late umbonato, carne alba; lamellis stipite adnexis, sinuatis, ventricosis, cinereis dein cinnamomeo-brunneis, acie alba; cystidiis clavato-ventricosis, membrana crassa praeditis, 30–70 x 15–30μ; sporis subovatis vel subellipsoideis, inequilateralibus, levibus, 10–20 x 6–8μ; stipite aequali, albo vel pileo concolore sed pallidiore, bulboso, ad apicem pruinoso.

In sand dunes or sandy places. Type specimens no. 22106, Herb. Cornell University, in sand dunes, Grand Haven, Mich., Sept. 23, 1907, W. T. Wallace, collector. In these specimens the pileus is pale ochre, and the stem white, becoming tinged with the color of the pileus. No. 18494, in sand, Sodus Point, Wayne Co., N. Y., B. C. Williams, collector (recd. Nov. 2, 1903); the pileus is wood-brown on the umbo, yellowish toward the margin, the margin in some splitting deeply. No. 24209, in sandy ground, Six Mile Creek, near Ithaca, N. Y., July 17, 1917, J. H. Faull, collector. These specimens are much smaller and the pileus walnut-brown to burnt umber (R. 1912). In nos. 18494 and 22106, the mycelium binds the sand into a large ball; the ball of sand in no. 24209 is much smaller, perhaps because the plants are much smaller.

This species is related to *Inocybe bulbosa* Pk. but differs in the character of the spores, cystidia, and bulb.

Inocybe atripes n. sp.

Solitaria vel gregaria, 2–6 cm. alta; pileo luteo-olivaceo dein fulvo-olivaceo vel umbrino, ovali, convexo dein expanso et subumbonato, 1.5–3.5 cm. lato, adpresse fibroso-squamoso vel subsquarroso, circum et in umbone frequenter areolato-rimoso; lamellis stipite adnexis, subellipsoideis, albidis dein olivaceo-luteis demum fulvo-olivaceis; cystidiis clavatis vel ellipsoideis, membrana crassa praeditis, 45–65 x 13–20μ; sporis subovatis vel subellipsoideis, inequilateralibus, levibus, 7–10 x 4.5–6μ; stipite velutino, deorsum umbrino vel nigro, sursum pallidiore, 2–5 mm. crasso.

Type specimens no. 19790, Herb. Cornell University, on ground in pine grove north side of Fall Creek, by Triphammer Falls, Ithaca, N. Y., June 24, 1906. H. H. Whetzel, collector.

This species has been collected several times in the vicinity of Ithaca, N. Y., during the last 15 years. It resembles *In. tenebrosa* Quélet (Asso. Fran. pl. 8, fig. 8, 1885) in external appearance, but differs in the velvety, non-striate stem. We have no knowledge of the cystidia in *In. tenebrosa*.

Inocybe brunnescens n. sp.

Gregaria, 4-6 cm. alta; pileo pallide brunneo dein umbrino, ovali vel convexo, obtuso, radiatim rimoso et partito, 4–5 cm. lato; lamellis stipite adnexis, avellaneis demum subumbrinis, acie cellis sterilibus et clavatis praedita; cystidiis nullis; sporis fabiformibus, levibus, 8–11 x 4.5–5.5μ; stipite albo, dein brunnescente, deorsum obscuriore, apice albido-pruinoso, 4–7 mm. crasso.

Type specimens no. 25020, Herb. Cornell University. Ground, open oak woods near sphagnum moor, about 2½ miles southeast of Oakland, Md., Sept. 16, 1917. G. F. Atkinson, collector.

Related to *In. fastigiata*, but differs in form, color, etc.

Inocybe cylindrocystis n. sp.

Gregaria, 4–7 cm. alta; pileo ochraceo, convexo dein expanso, subumbonato, fragili, fibrilloso-squamuloso, versus marginem repando et partito, 1–3 cm. lato, umbone levi; lamellis stipite adnexis, ellipsoideis; cystidiis numerosis, cylindricis, curtis, membrana tenella praeditis, 30–35 x 9–11μ; basidiis 25–27 x 7–8μ; sporis obovatis vel subellipsoideis, inequilateralibus, levibus, 9–10 x 4–5–6μ; stipite fibroso-striato, contorto, aequali, leniter fibrilloso, sursum pruinoso, albo dein luteo leniter tincto, 4–5 mm. crasso.

Ground, McGowan's woods, near Ithaca, N. Y., July 14, 1903. H. S. Jackson, collector. Type specimens no. 15211, Herb. Cornell University.

Related to *Inocybe hirtella* Bres., but differs in spores and cystidia.

Inocybe fastigiella n. sp.

Solitaria vel gregaria, 5–6 cm. alta; pileo umbrino, campanulato dein expanso, prominenter umbonato, demum radiatim rimoso, circum umbonem leniter fibrilloso; lamellis stipite adnexis, angustis, ellipsoideis, acie alba; cystidiis nullis sed acie lamellarum cellis clavatis praedita; sporis subreniformibus, levibus, 7–9 x 4–5μ; stipite aequali, fibrilloso-striato, intus albo, basi subbulbosa.

On ground, Cascadilla woods, Campus, Cornell University, Ithaca, N. Y., Aug. 12, 1908. E. J. Petry, collector. Type specimens no. 22525, Herb. Cornell University.

Inocybe leptocystella n. sp.

Solitaria vel gregaria, 6–7 cm. alta; pileo umbrino, convexo dein leniter expanso, demum umbonato, adpresse-squamoso, margine partito, 12–15 mm. lato; lamellis stipite adnexis, ventricosis, pallidis dein umbrinis, acie alba; cystidiis subcylindricis, pedicellatis, membrana tenui praeditis, 45–60 x 10–16μ; sporis subovoideis, inequilateralibus, levibus, 8–11 x 5–7μ; stipite subflexuoso, fistuloso, aequali, umbrino, fibrilloso-squamoso, apice pruinoso atque albo, 2–3 mm. crasso.

On leaf mold in wet ground, Michigan Hollow Swamp, near Danby, N. Y., July 12, 1906. C. W. Edgerton, collector. Type specimens no. 19844, Herb. Cornell University. Related to *In. carpta* and *In. leptocystis*, but differs from the former in being less scaly and in the character of the cystidia, from the latter in color and other external characters.

Inocybe leptocystis n. sp.

Gregaria et dispergens, 3–7 cm. alta; pileo brunneo, 1–3 cm. lato, campanulato vel convexo, dein expanso et umbonato, sericeo, leniter adpresse-squamuloso vel levi, carne alba; lamellis stipite adnexis, angustatis, ellipsoideo-linearibus, confertissimis, griseis dein arellaneis; cystidiis numerosis, cylindricis vel cylindrico-capitatis, vel subventricosis, pedicellatis, membrana tenui praeditis, 50–65 x 12–16μ; sporis subellipsoideis, inequilateralibus, levibus, 7–9 x 4–5μ; stipite albido vel carneo tincto, solido, leniter fibrilloso, 2–5 mm. crasso, apice pruinoso, basi albo.

Ground, Cascadilla woods, Campus, Cornell University, Ithaca, N. Y., July 14, 1903. Charles Thom, collector. Type specimens no. 15209, Herb. Cornell University.

Inocybe leptophylla n. sp.

Solitaria vel gregaria, 2–4 cm. alta; pileo brunneo vel umbrino, frequenter purpureo tincto, 1–2.5 mm. lato, subsquarroso-squamoso, versus marginem fibrilloso; lamellis stipite adnexis, ellipsoideis, cinnamomeis vel fulvo-olivaceis; acie lamellarum concolore et basidiis et cellis sterilibus praedita; cystidiis nullis; sporis subglobosis vel leniter elongatis, prominenter et radiatim nodulosis, 7–11 x 6–8μ; stipite subconcolore, fibrilloso et subsquamuloso, deorsum fuliginoso, sursum pallidore, 2–3 mm. crasso.

On ground and on very rotten wood, Ithaca, N. Y. Type specimens no. 13370, Coy Glen, near Ithaca, N. Y., Aug. 7, 1902. W. Bradfield, collector. Herb. Cornell University. Several other collections have been made of this species.

Related to *In. lanuginosa*, but differs in the absence of cystidia.

Inocybe leptophylla var. **cystomarginata** n. var.

Gregaria, 2–3 cm. alta; pileo brunneo, purpureo-tincto, subsquarroso-squamoso, 1–1.5 cm. lato; lamellis stipite adnexis, cinnamomeis vel fulvo-olivaceis, acie lamellarum cystidiis subampullaeformibus, 35–50 x 12–15μ, praedita, in superficiem lamellarum cystidiis nullis; sporis prominenter noduloso-angulatis, 9–11 x 7–9μ; stipite pileo concolore, fibrilloso-squamoso, 2–3 mm. crasso.

Ground, woods, border of cliff, north side of Fall Creek, near Forest Home, N. Y., July 8, 1917. J. H. Faull, collector. Type specimens no. 24164, Herb. Cornell University.

This may prove to be worthy of specific rank. It differs from *In. lanuginosa* in its different cystidia and their presence only on the edges of the lamellae.

Inocybe longicystis n. sp.

Solitaria, 4–5 cm. alta; pileo atro-brunneo, in centrum squarroso, 2 cm. lato, carne alba demum brunneo tincta; lamellis stipite adnexis, angustis, ellipsoideis, griseis dein brunneo tinctis; cystidiis subcylindrico-ventricosis vel subampullaeformibus, frequenter subcapitatis, in aciem lamellarum frequenter elongatissimis, 50–70 x 15–20μ; sporis subglobosis vel leniter elongatis, prominenter substellato-nodulosis, 7–10 x 6–8μ; stipite brunneo, ad apicem pallido et fibrilloso, ad basem squamoso, solido, 5 mm. crasso; carne alba tarde brunneo tincta.

On leaf mold on hillside in mixed forest, Endogone Ravine, Seventh Lake, Adirondack Mts., N. Y., Aug. 13, 1917. F. C. Stewart, collector. Type specimens no. 24321, Herb. Cornell University.

Inocybe marmoripes n. sp.

Solitaria vel subcespitosa, 5 cm. alta; pileo ochraceo-fulvo, fibrilloso-squamoso, 2–2.5 cm. lato; lamellis stipite adnatis, olivaceis; acie lamellarum albida et cellis clavatis praedita; cystidiis nullis; sporis angustis, elongatis, subellipsoideis et inequilateralibus, levibus, 8–13 x 3.5–5μ; stipite ochraceo-fulvo, fibrilloso, demum irregulariter transversim marmorato-squamoso, 6 mm. crasso.

Ground, woods west of Cayuga Lake, near Glenwood (suburb of Ithaca), N. Y., July 30, 1917. Leva B. Walker, collector. Type specimens no. 24275, Herb. Cornell University.

Inocybe nigrescens n. sp.

Gregaria, 4–6 cm. alta; pileo campanulato vel convexo dein expanso, umbonato, 1.5–3 cm. lato, pallide-brunneo, adpresse-fibrilloso-squamoso, versus marginem interdum leniter rimuloso; lamellis stipite adnexis, ellipsoideis, angustatis, avellaneis dein argillaceo-luteis; cystidiis cylindricis vel subventricosis, subpedicellatis vel nonpedicellatis, membrana moderatim crassa praeditis, 50–75 x 12–16μ; sporis subglobosis vel leniter elongatis, prominenter radiatim nodulosis, 9–11 x 8–10 vel 11 x 8μ; stipite pallide-argillaceo-luteo, bulboso, apice pruinoso, deorsum leniter velutino, siccato nigrescente, 3–4 mm. crasso.

Ground, woods, near Ithaca, N. Y., July 25, 1917. H. E. Stork. Type specimens no. 24260, Herb. Cornell University. On drying the entire plant becomes much darker, the stem black. Related to *In. asterospora*, but distinguished by the very slight rimose condition of the pileus, the subvelvety stem, blackening as it dries, etc.

Inocybe ochraceoscabra n. sp.

Gregaria vel dispergens, 3–4 cm. alta; pileo fulvo-ochraceo, 2–4 cm. lato, convexo dein expanso, subumbonato vel gibboso, adpresse-fibrilloso-squamuloso, umbone frequenter areolato-rimoso et margine repando et partito, carne alba; lamellis stipite adnatis, emarginatis, albis dein avellaneis, acie albida; cystidiis clavato-ventricosis, pedicellatis, membrana crassa praeditis, 45–60 x 15–25μ; sporis elongatis, leniter noduloso-angulatis, 9–12 x 5–7μ; stipite aequali vel deorsum tenuiore, 3–5 mm. crasso, brunneo, apice pruinoso, exteriore membrana tenui, albido, fibrillosa praedito, carne brunnea.

On grassy ground under young white pine and near hard maple trees, Professor Tichener's lawn, Cornell Heights, Ithaca, N. Y., Aug. 12, 1912. G. F. Atkinson, collector. Type specimens no. 23366, Herb. Cornell University.

Related to *In. decipiens* Bres., but differs in color and character of the stipe, smaller spores, etc.

Inocybe olpidiocystis n. sp.

Gregaria, 5–7 cm. alta; pileo pallide luteo, in centro brunneo, convexo-depresso, margine repando et partito, 4–6 cm. lato; lamellis stipite adnexis, late sinuatis, ventricosis, 8–10 mm. latis, griseis dein subferrugineis; cystidiis ovato-ventricosis, non pedicellatis, membrana crassa praeditis 40–60 x 15–30μ; sporis subellipsoideis vel subovatis, inequilateralibus, levibus, 9–13 x 6–8μ; stipite albo, fibrilloso-striato, 1–1.25 cm. crasso.

In grassy ground, President White's lawn, Campus, Cornell University, September 22, 1902. W. Bradfield, collector. Type specimens no. 13745, Herb. Cornell University.

Inocybe paludosella n. sp.

Solitaria vel gregaria, 5 cm. alta; pileo campanulato, expanso, dein prominenter umbonato, demum leniter rimosulo, luteo-brunneo, 1.5–2 cm. lato; lamellis stipite adnexis, ellipsoideis, albis, demum ochraceo-fulvis; cystidiis clavato-ventricosis, 45–70 x 12–20μ; sporis elongatis, leniter nodulosis, 8–12 x 6–7μ; stipite albo, cavo, glabro, basi bulbillo rotundo praedito, 2.5 mm. crasso.

On rich leaf and wood mold in swamp, mixed woods of red spruce, beech, maple, etc., Seventh Lake, Adirondack Mts., N. Y., Aug. 16, 1917. F. C. Stewart, collector. Type specimens no. 24320, Herb. Cornell University.

Inocybe retipes n. sp.

Solitaria, 7 cm. alta; pileo brunneo, 2 cm. lato, campanulato, umbonato, versus marginem sericeo et radiatim fibroso, leniter radiatim rimuloso, circum umbonem adpresse-fibroso-squamoso, cuticula umbonis rimosa; lamellis stipite adnatis, avellaneis dein pallide-brunneis, acie crenulata; cystidiis cylindrico-ventricosis, pedicellatis, membrana crassa praeditis, 60–75 x 14–18μ; sporis ovatis, inequilateralibus, levibus, 8–11 x 4–6μ; stipite solido, intus albo, aequali, 3 mm. crasso, pileo concolore, fibrilloso-reticulato; reticulo obscuro-brunneo, interstitia pallidiora habente; basi bulbilloso.

Swampy ground by stream, mixed woods of red spruce, maple, beech, etc., Seventh Lake, Adirondack Mts., Aug. 13, 1917. G. F. Atkinson, collector. Type specimen no. 24319, Herb. Cornell University.

Inocybe rubellipes n. sp.

Gregaria, 3–6 cm. alta; pileo luteo-ochraceo, campanulato dein obtuso-umbonato, 1–1.5 cm. lato, adpresse-squamuloso, in centro frequenter areolato-rimoso, versus marginem leniter rimuloso, carne albida; lamellis stipite adnexis, angustatis, confertissimis, avellaneis, fractis rubescentibus; cystidiis numerosis, cylindricis, rarenter subventricosis, pedicellatis, 55–80 x 12–18μ; sporis ovalibus, inequilateralibus, levibus, 7–12 x 5–7μ; stipite 3–4 mm. crasso, pileo concolore sed pallidiore, basi albo, fracto rubescente.

Ground, woods, Fall Creek Gorge, Ithaca, N. Y., June 21, 1911. B.B. Higgins, collector. Type specimens no. 23042, Herb. Cornell University.

Related to *In. trinii*, but differs in color of the pileus and stem being somewhat yellowish, different spores, and cystidia, pileus not becoming reddish, etc.

Inocybe sambucella n. sp.

Gregaria vel dispergens, 4–5 cm. alta; pileo albo, convexo dein expanso, subrepando, levi, 2–2.5 cm. lato, cortice cellis magnis

praedito, cuticula pilei fibrillis veli universalis probabiliter facta; lamellis stipite adnexis, avellaneis; cystidiis subellipsoideis vel clavatis vel subventricosis, membrana crassa praeditis, 40–50 x 11–15μ; sporis subovalibus vel suboblongis, levibus, 7–10 x 4–6μ; stipite albo, 4–5 mm. crasso, fibrilloso-striato, sursum pruinoso.

Ground, Cascadilla woods, Campus, Cornell University, Ithaca, N. Y., Nov. 4, 1905. C. W. Edgerton, collector. Type specimens no. 19579, Herb. Cornell University.

Inocybe submuricellata n. sp.

Solitaria, 4–5 cm. alta; pileo pallide-ochraceo, campanulato dein expanso et umbonato, tenuiter fibrilloso-squamuloso, 2–3 cm. lato; lamellis stipite attenuato-adnexis, pallide-luteis, acie albida; cystidiis numerosis, cylindricis vel cylindrico-ventricosis, pedicello frequenter longissimo, membrana crassa praeditis, 50–100 x 10–24μ; sporis ovalibus, subreniformibus, inequilateralibus, levibus, 8–12 x 5–7μ; stipite pileo concolore sed pallidiore, fibroso-striato, fibrilloso, sursum pruinoso, 3–4 mm. crasso.

On decaying leaves of conifers, Coy Glen, near Ithaca, N. Y., Oct. 29, 1902. C. H. Kauffman, collector. Type specimens no. 14180, Herb. Cornell University.

Related to *In. muricellata* Bres. Ann. Myc. 3: 160, no. 1905, but differs in having delicate fibrillose scales, not truly scaly, adnexed lamellae, stipe not marginate bulbous, etc.

Inocybe subrubescens n. sp.

Solitaria vel gregaria, 4–6 cm. alta; pileo subochraceo demum leniter rubescente, 1.5–2.5 cm. lato, campanulato, expanso dein umbonato, prominenter squamoso, margine leniter squamuloso; lamellis stipite adnexis, ellipsoideis, umbrinis, acie lamellarum alba et cellis clavatis praedita; cystidiis nullis; sporis subreniformibus, levibus, 12–17 x 6–8μ; stipite aequali, subochraceo, demum rubescente, apice scabriusculo, versus basim sparsim velutino, solido, carne alba demum subrufescente.

On ground in a small wood north side of Fall Creek, Ithaca, N. Y., Oct. 16, 1909. Adeline Ames. Type specimens no. 22717, Herb. Cornell University.

Resembles *In. scabra*, but differs in absence of cystidia, reddish color, larger spores, etc.

Inocybe tenerrima n. sp.

Gregaria vel dispergens, 2–3 cm. alta; pileo conico, umbonato, rubello-brunneo, subfibrilloso-squamoso, carne non rubescente, 3–4

mm. alto et lato; lamellis avellaneis dein rufo-brunneis, adnato-adnexis, ventricosis, acie lamellarum cellis clavatis vel subventricosis praedita; sporis subreniformibus, levibus, 9–15 x 6–8μ; stipite pileo concolore, fibrilloso, solido, apice albo et pruinoso, carne alba non rubescente.

Ground in woods south of Michigan Hollow, near Danby, N. Y., Aug. 4, 1917. J. H. Faull, collector. Type specimens no. 24285, Herb. Cornell University.

Related to *In. hirsuta* and *In. calamistrata*, but differs from the former in size, in stipe not greenish at base, lamellae ventricose, etc., and from the latter in stipe not blue at base; from both in scales not squarrose but lanuginose.

Inocybe tubarioides n. sp.

Gregaria, 3–5 cm. alta; pileo convexo dein expanso, hygrophano, pallido-castaneo, minute fibrilloso-squamosa, 6–12 mm. lato; lamellis concoloribus, subtriangularibus, dente decurrentibus, acie albida; cystidiis subcylindricis vel subclavatis, 60–75 x 12–16μ; sporis noduloso-angulatis, obovatis vel subquadratis, 6–9 x 4–6μ; stipite concolore, fibrilloso.

On very rotten wood, McGowan's woods, near Ithaca, N. Y., July 17, 1903. C. H. Kauffman, collector. Type specimens no. 15238, Herb. Cornell University. Other collections are no. 15294, on very rotten wood, McGowan's woods, July 21, 1903, H. S. Jackson, collector; no. 18350, on very rotten wood, McGowan's woods, July 13, 1904, H. S. Jackson.

Superficially the plants resemble *Tubaria furfuracea*.

Inocybe ventricosa n. sp.

Gregaria, 3–4 cm. alta; pileo convexo-expanso dein leniter gibboso, 1–2.5 cm. lato, luteo-ochraceo, margine subrimuloso; lamellis stipite adnexis, subluteis; cystidiis ellipsoideo-clavato-ventricosis, membrana crassa praeditis 40–55 x 12–18μ; sporis leniter noduloso-angulatis, 6–9 x 5–6μ; stipite luteo-subochraceo, aequali, vel basi interdum leniter incrassato, leniter albo-velutino, apice praecipue pruinoso, deorsum leniter fibrilloso, 2–3 mm. crasso.

On ground near Beebe Lake, Aug. 11, 1903. W. Bradfield, collector. Type specimens no. 13453, Herb. Cornell University.

Inocybe violaceoalbipes n. sp.

Gregaria vel subcespitosa, 4–4.5 cm. alta; pileo pallide ochraceo-fulvo, convexo dein expanso, subgibboso, prominenter squamoso et

irregulariter rimoso, versus marginem leniter radiatim rimuloso, 2–2.5 cm. lato; lamellis late sinuato-adnexis, ventricosis, isabellinis demum ferrugineo-fulvis; cystidiis cylindricis vel ventricosis, membrana crassa praeditis, 50–70 x 10–16 μ; sporis ovalibus vel subreniformibus, inequilateralibus, levibus, 8–10 x 5–6 μ; stipite 4–5 mm. crasso, deorsum albo, sursum violaceo, minute albido-velutino, non squamoso, basi bulbosa cuius volva membranea interdum se liberat; deorsum carne alba, sursum violacea.

Ground, Cascadilla woods, Campus, Cornell University, Ithaca, N. Y., Oct. 3, 1907. E. J. Petry, collector. Type specimens no. 22171, Herb. Cornell University.

Related to *In. obscura* and *In. cincinnata.*

Inocybe virgata n. sp.

Solitaria vel gregaria, 5–8 cm. alta; pileo campanulato dein expanso et umbonato, levi, 1.5–2.5 cm. lato, radiatim fibroso et leniter rimoso, atro-brunneo, versus marginem pallidiore et virgato; lamellis stipite adnatis, sinuato-uncinatis, subdistantibus, albis, demum fulvo-olivaceis, acie albida; cystidiis ventricosis vel clavato-ventricosis, pedicellatis, membrana moderatim crassa praeditis, 40–65 x 15–20 μ; sporis ovalibus vel ovato-subellipsoideis, vel fabiformibus, levibus, 7–10 x 5–6 μ; stipite concolore, sursum pallidiore, basi albo-mycelcoideo, 3–4 mm. crasso, apice albo-pruinoso, carne brunneo tincta.

On bare wet ground or leaf mold in swamps. No. 25079 (type specimens, Herb. Cornell University) on bare ground in a spruce moor by Millers Run, beyond Hoop Pole Ridge, near Oakland, Md., Sept. 27, 1917. G. F. Atkinson, collector. No. 25063 on leaf mold in low swampy ground along stream near Boiling Spring, a few miles from Deer Park, Md., Sept. 21, 1917. G. F. A.

A number of the above described species have, for several years, received provisional MS. names as a matter of temporary convenience. Several of these have been changed to more suitable names. Unfortunately two of these provisional names were published through inadvertence.[1] Since the descriptions could not well be drawn from the dried Michigan plants, it seems desirable not to employ those *nomina nuda*, and more appropriate names are here used in their stead. *In. entomospora* becomes *In. leptophylla* and *In. scabroides* becomes *In. leptocystis*, unless the Michigan plants should prove to be specifically distinct from these two described here.

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[1] See Kauffman, C. H. Unreported Michigan Fungi, p. 34. Michigan Acad. Sci. 8: 26–37.

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THE GEOGRAPHIC AFFINITIES OF THE VASCULAR FLORAS OF NEW ENGLAND, THE MARITIME PROVINCES AND NEWFOUNDLAND[1]

M. L. FERNALD

The region assigned me for discussion, the area east of the Hudson, Champlain and Richelieu Valleys and south of the St. Lawrence and the Straits of Belle Isle, including the political areas of New England, southeastern Quebec, the Maritime Provinces and Newfoundland, has long been recognized by the geologist and the physiographer as essentially an orographic unit. Exhibiting the highest degree of complexity in its geological history and structure, as contrasted with the essentially uniform structure of vast areas in the interior of the continent, the region may be defined as the northeastern extension of the Appalachian system, bordered on the extreme south, about the southwestern shores of the Gulf of St. Lawrence, and inland along the principal valleys by level plains which were largely occupied by the Champlain sea at the close of the Pleistocene. In fact, so generally was the region affected by the Wisconsin glaciation and the Champlain subsidence, that only a few very isolated localities seem to have escaped the general extermination of the flora which had formerly occupied the land. We have consequently to deal in this region with a flora which has migrated to its present position since the close of the Pleistocene glaciation. The attempts to account for these migrations and to trace with approximate accuracy the geographical history and wanderings of the various components of the complex which we now call the indigenous flora of the region are fascinating and vastly important problems, but without a thoroughly accurate knowledge of the

[1] Presented at the joint session of the Systematic Section of the Botanical Society of America and the American Fern Society at Pittsburgh, 31 December, 1917.

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flora as it now exists all such explanations and attempts at correlation are futile; and, although I have been allowed the maximum time to present my case, I must emphasize at the start the impossibility of presenting in one hour more than the briefest suggestion of the problem, leaving until we better understand our flora the consideration of its exact geographic origin.

This limited region, from the Hudson and Champlain Valleys to the Straits of Belle Isle, contains only about 200,000 square miles of land and fresh water, far less than the state of Texas, and approximately the area of the combined states of Colorado and Wyoming. In latitude the region lies chiefly between the 41st and 50th degrees—or parallel with the region from southern Iowa to Lake Winnipeg or from Humboldt County, California, to southern British Columbia. Among the earliest districts in America to be settled by Europeans and the seat of many of our ancient institutions of learning, the region, one might naturally suppose, would ere this have had its flora thoroughly worked out. In fact more than one botanist resident outside New England and some who have lived within her borders have expressed this belief. Thus we find the printed statement of one who has attempted an exposition of all the phytogeographic areas of the continent, that "no one region in North America has been more carefully studied botanically than New England."

Nevertheless, during the past quarter-century, since active botanical exploration of New England, adjacent Canada and Newfoundland has been prosecuted by the present generation, many hundreds of species have been added to the known flora of the region. And during the last decade it has been a poor summer indeed which has not yielded to light a score of novelties, while exceptional seasons have yielded a full hundred additions to the known vascular flora of the area. Certain days stand out vividly in my mind, when the additions to the flora for the single day have mounted to fifteen and sometimes even to twenty-five species.

The majority of plants of the greatest phytogeographic interest are, naturally, species of highly specialized requirements and consequently somewhat localized in a region. They are not to be seen from the stage-coach, steamboat or railroad-train but must be sought in their exclusive haunts. It is for this reason that many easy-going botanists have entirely missed the truly significant plants of regions they have glimpsed from the steamboat or train. For instance, when

the American Association met at Montreal in 1882, an excursion was made down the St. Lawrence and up the Saguenay. In writing of this excursion through one of Nature's botanic gardens, an active botanist of that time said: "Probably the prevailing feeling among botanists at Montreal, from 'The States,' was one of surprise and disappointment that the Canadian flora was so familiar. At Montreal I noticed nothing of interest either among the weeds or the wild flowers. At Quebec, *Euphrasia officinalis* was abundant on the ramparts. At Tadousac, *Empetrum nigrum* and *Vaccinium Vitis-Idaea* were growing at sea-level, the latter so abundant that children were bringing in pails of the berries for sale.

"At Ha! Ha! Bay, where I had intended stopping if the flora seemed attractive, the only unfamiliar plant was *Senecio vulgaris* as an abundant weed.

"The meeting next year at Minneapolis will doubtless offer many more botanical attractions to eastern botanists."

Now, to one who has tramped the shores and clambered with the aid of an alpine rope over many cliffs of the lower St. Lawrence it is apparent that the writer of the passage above quoted was merely the prototype of that later group of botanists whose depth of interest in the problems of phytogeography finds expression in the statement that "it would be quite possible to prepare a fairly satisfactory description of the vegetation of a given region without naming a single species." From this superficial and uncommunicative point of view the traveller down the St. Lawrence might recognize and, if in a communicative mood, perhaps even enumerate such trees as *Abies balsamea*, *Picea canadensis*, or *Acer rubrum* and make a sort of guess as to which mountain ash, white birch, or poplar lined the shores, and he would be reasonably safe in identifying *Heracleum lanatum* and *Sambucus racemosa*; but not one of these wide-spread and almost ubiquitous plants would give him the faintest indication of the botanical interest of the region. If, however, he overcame the inertia of travel sufficiently to walk three minutes from the wharf at Rivière du Loup, the last stop of the steamer before crossing to the Saguenay, he would discover *Cornus suecica*, an arctic species here reaching one of its southernmost outposts in America, *Osmorhiza divaricata* of southern British Columbia, Washington and Oregon, *Arabis Drummondi*, var. *connexa* of the Rocky Mountains, *Poa eminens* of Alaska and adjacent Asia, and scores of other species whose presence here at once suggests the most far-reaching phytogeographic problems.

The localization of plants in the region and the impossibility of recognizing the most significant of them from the steamboat, stage-coach or railroad-train, is further emphasized by a statement of that prince of New England explorers, William Oakes, whose experience elsewhere should have taught him to be more cautious in his drawing conclusions. Writing to his friend Dr. J. W. Robbins on August 14, 1828, Oakes said: "The greater part of July I have spent 'down East' even as far as Quoddy Head which lieth more eastward than Eastport. I have seen there however but few plants new to N. E. and am convinced that no great accessions to the N. E. Flora, and of absolutely new plants hardly any, are to be expected from the State of Maine." For this reason, apparently, Oakes, who had visited one of the most sterile corners of the state, thereafter avoided the supposedly barren state of Maine, the home of the famous crops of Aroostook potatoes, and thus missed some hundreds of species which there make up an essential element of the New England flora. Even at barren Quoddy Head, where Oakes *did* explore, he failed to detect *Iris setosa*, then known only from Siberia; *Comandra livida* and *Carex norvegica*, Arctic species at that time unknown in New England; and the characteristic little *Euphrasia purpurea*, subsequently discovered and described from Newfoundland.

These illustrations should be sufficient to indicate my point of view, that, although the dominant and more or less ubiquitous species may serve for the major phytogeographic divisions of a continent, they are of little value in the more refined studies of plant distribution; but that it is the relic species now localized in isolated areas which give us clues to the long cycles of plant migrations—marches and countermarches—which have accompanied the different geological epochs since the early Cretaceous; and it is to these relic colonies, both of plants and of animals, that the historical geologist must turn in the reconstruction of ancient lands now quite obliterated or buried beneath the great oceans. And even if we belong to that unimaginative group of botanists who would completely divorce taxonomy from other fields of science, we must at least recognize that the discovery in the indigenous flora of eastern Quebec of plants described from Montana, Alaska, or Siberia, or in Alberta or Denmark of species first detected about the Gulf of St. Lawrence, forces upon us the necessity for caution in characterizing new species. It has been an easy principle of convenience but of very unsound scholarship among

us to assume that a novelty found, let us say, in Alberta, must inevitably be an undescribed species, quite overlooking the fact that the identical species may have been already described from Siberia or from Newfoundland. Sound taxonomic work, therefore, demands a broader and more accurate insight into phytogeographic laws, and it is with the hope that by mutual comparisons we may come to a clearer understanding of the relationships of our complex floras that I look with special satisfaction upon the formation of this new section of the Botanical Society. From this long peroration you will see that I have a double motive in presenting for your consideration some of the more patent facts brought out in studying the geographic affinities of the flora with which I am most familiar.

As I have already said, the area I am sketching consists of approximately 200,000 square miles of land, ranging in character from the most arable farm-lands of the Aroostook, Connecticut and Champlain Valleys to sandy wastes, Hudsonian tundra, subalpine forests, saline marshes, granitic rockfields, limestone barrens and seacliffs, and arid cañons. These and scores of other distinct habitats make up a region in many parts quite unexplored and unmapped, but with a phenomenally extensive indigenous flora. The area covered by Coulter and Nelson's *Rocky Mountain Flora*, from northern Arizona and New Mexico to the Black Hills, Montana and southern Idaho, includes about 480,000 square miles and has, as recognized in that work, an indigenous flora of 2,836 species and geographic varieties. Our northeastern region, with an area of 200,000 square miles, less than half Coulter and Nelson's area, has a known indigenous flora as extensive as theirs, more than 2,800 species and varieties; and of these more than 250 are strictly endemic while an additional 50 overstep the bounds of the region only by occurring on Long Island, the Adirondacks, or in southern Labrador. This endemic or essentially endemic element, making altogether more than 10 percent of the flora, is well illustrated by *Rosa nitida* (fig. 1) of the acid bogs from Newfoundland to eastern Connecticut.

Most conspicuous to the casual observer are, of course, the common trees, shrubs and widely dispersed herbs. These, for the most part, are species of broad and continuous range throughout the Alleghenian, Canadian or Hudsonian districts, and often beyond. Typical illustrations of these common and widespread species are the native red currant, *Ribes triste*, and the balsam fir, *Abies balsamea*, of

broad Hudsonian and Canadian range nearly across the continent. Other more familiar examples, because of more southern range, of these widely dispersed species of nearly continuous distribution over a vast area, are *Clematis virginiana*, extending in abundance from eastern Quebec to Georgia and Lake Winnipeg; and *Eupatorium perfoliatum*, abounding from Prince Edward Island to Florida, Louisiana and the Dakotas. The entrance of these floras into the New England-Maritime Province region in solid phalanx from the extensive regions to the southwest, west, northwest and north presents no problem and this major element of our flora (the common and widespread plants) may be dismissed with this brief mention, although such plants as these are the ones most emphasized by many phytogeographers. Similarly we may pass the more strictly Alleghenian plants, such as *Ilex monticola* (fig. 2), which cling conservatively to the rich wooded slopes of the Alleghenies and in New England are found chiefly on the northern extension of the Alleghenies, the Taconics of western Massachusetts and Connecticut.

Of greater interest are the coastal plain species, because they represent in New England, eastern Canada and Newfoundland a relic of the extensive flora which during the late Tertiary migrated northward along the then highly elevated continental shelf and at the drowning of the shelf were left as relics at isolated points. This isolated remnant of the flora derived from the southern coastal plain is represented by about 200 species north of New Jersey, and nearly every excursion to southwestern Rhode Island, Cape Cod, Plymouth County (Massachusetts), Nantucket, southern Nova Scotia, Cape Breton, eastern New Brunswick, Prince Edward Island, the Magdalen Islands or southeastern Newfoundland, adds to the number of thus isolated species known to us or extends our knowledge of those already recognized.

Some of these range northward only from New Jersey, Delaware or Maryland, such species as *Eriocaulon Parkeri*, isolated in the brackish estuaries of the Potomac, Delaware, Housatonic, Mill River (Conn.), Merrimac, Kennebec and Penobscot; or *Chrysopsis falcata*, the common "yellow aster" of southern New England. Others extend north from Florida, Mississippi or southeastern Texas, such species as *Ilex glabra* or the genus *Bartonia* (fig. 3); while a number, like *Drosera filiformis* (fig. 4), occur in the Northeast as colonies quite isolated from the South. Some, like *Panicum Wrightianum*, were originally de-

scribed from the Antilles; while others, like *Eleocharis interstincta* (fig. 5) or *Erigeron pusillus*, are widely dispersed in tropical and subtropical America, occurring in the Bermudas and tropical Mexico, and by way of the Antilles or of Central America extending to South America.

Swinging northward from the Gulf of Mexico along the Mississippi basin, we come into a flora which is familiar to the New Englander, though rarely known to the botanist of the South Atlantic States. This flora common to the Mississippi basin and southern New England is well illustrated by *Ludvigia polycarpa* (fig. 6), which occurs in sloughs and wet depressions from southwestern Ontario and Ohio to Nebraska, southern Missouri and Tennessee,[2] and east of the Appalachians occurs in three isolated areas: Cumberland Co., Maine; Middlesex Co., Massachusetts; and Hartford Co., Connecticut. This group of species is further illustrated by *Cyperus Engelmanni*, a plant of less general occurrence in the Great Lake-Mississippi region—from southern Ontario to Minnesota and Missouri—and eastward found only in Seneca Co., New York, and in Middlesex Co., Massachusetts, where it has long been known as a characteristic plant of lake-alluvium.

The plants of the drier prairies and plains of the interior are not so definitely restricted to the interior of the continent as might be supposed. In fact, many lists of characteristic plants of dry prairies have a very familiar appearance to the New Englander—*Sporobolus heterolepis*, *Sorghastrum nutans*, *Andropogon furcatus*, *Muhlenbergia mexicana*, *Aster novae-angliae*, *Heliopsis scabra*, etc. These plants, typical of the drier prairies and plains of the interior, are well illustrated by *Solidago rigida*, which is widely dispersed from the Mississippi Valley westward, in one or another of its variations, to the Rocky Mountains and northward to Peace River, and eastward into Ohio and western New York. East of the Alleghenies the plant is localized, from the District of Columbia to Massachusetts.

Another characteristic element in the western flora which has a greater representation in the extreme East than is generally realized is the flora of subsaline or brackish habitats of the Great Plains and the foothills of the Rocky Mountains; such plants, for instance, as *Erigeron loncophyllus*, a species of saline meadows from the Black

[2] In the maps the northeastern range may be taken as fairly representing the facts in detail, but the ranges west and south of New England are only approximate, and, owing to lack of detailed reports, cannot be considered final.

Hills and the Saskatchewan Plains to Oregon and California, reappearing in northern Asia, and on Anticosti Island at the mouth of the St. Lawrence. A very similar distribution is shown by the Section *Conyzopsis* of the genus *Aster*, a unique group of annual essentially rayless plants with three species: the widely dispersed *A. angustus* of the Great Plains of western North America, salt plains of southern Siberia and Afghanistan and shores of the lower St. Lawrence; a second species, *A. frondosus*, of alkaline spots from the Rocky Mountains to the Pacific; and a third species, *A. laurentianus*, known only from saline or brackish sands of the Gulf of St. Lawrence. A very similar range is shown by several aquatic plants of which a good illustration is *Potamogeton filiformis*, var. *Macounii* (fig. 7), widely spread from the southwest side of Hudson Bay to Alberta and southern California, but eastward known only from Prince Edward Island and the Magdalen Islands, where it is a highly characteristic plant. In fact, just as recent botanizing on Cape Cod and Nantucket is taking much of the distinctive lustre from the botanical fame of the New Jersey Pine-barrens, so the exploration of the saline sands of the lower St. Lawrence, Prince Edward Island and the Magdalen Islands is gradually adding to our known flora of the Northeast a large proportion of the plants of the wet areas of the Great Plains and saline prairies.

So much, very briefly, for the temperate American affinities of the New England-Maritime Province-Newfoundland flora. Now turning to the boreal affinities, we have, of course, an extensive Hudsonian flora, already mentioned, which extends almost uninterruptedly from the Barren Lands and the Labrador Peninsula to northern New England; but in case of the boreal as with the temperate floras the greatest phytogeographic interest attaches to the species of discontinuous range. The most familiar examples of discontinuous ranges in our arctic-alpine flora are, naturally, the widely dispersed circumpolar types, such as *Saxifraga oppositifolia*, of broad range across Arctic Europe, Asia and America, extending locally southward to favorable alpine or subalpine habitats, in America the limestones of western Newfoundland, Anticosti, Gaspé and the northern Green Mountains in the East, the northern Rocky Mountains in the West; or *Salix reticulata* of similar occurrence in the Arctic, but in America extending southward very locally only to western Newfoundland, James Bay, and southern Alaska. Of much more restricted range in the North are the Greenland-Labrador types, many of which, like

Arenaria groenlandica, extend to the New England mountains and coast. But these, like the circumpolar species, would naturally be expected.

The most surprising feature of our alpine and subalpine flora and one which was hardly realized until recent years is the great number of species which are more typical of the Rocky Mountains, Alaska, or even of the northern Sierra Nevada. In the Gaspé Peninsula of Quebec, for instance, a region with an indigenous flora of 1,200 species, three fourths of the species, 800, are plants which occur also in the northwestern United States, British Columbia or Alaska; while other regions in our area considerably extend the number. It is not surprising, therefore, to find along the smaller streams among the Gaspé mountains such characteristic Cordilleran plants as *Lonicera involucrata* (fig. 8) or *Osmorhiza obtusa*, or on the limestone gravels such typical species of the Canadian Rocky Mountains as *Dryas Drummondii* (fig. 9) or *Salix vestita*.

A still more northwestern flora is represented by such plants as *Adiantum pedatum*, var. *aleuticum*, which extends from the Sierra Nevada of California very locally eastward into the Rocky Mountains, thence northwestward along the Coast Range to the Aleutian Islands, and on to northern Japan; known in the east only from the serpentine mountains of southeastern Quebec and Newfoundland. An even more distinctively northwestern species is *Vaccinium ovalifolium* of Washington, British Columbia and Alaska, which reappears about Lake Superior, and again on the Gaspé Peninsula of Quebec, in Newfoundland and adjacent Labrador.

The maritime flora also shows a large North Pacific element, such plants as *Arenaria peploides*, var. *maxima*, occurring on the shores of Japan, Kamtchatka and the Aleutian Islands, and again in western Newfoundland; while a strong Bering Sea affinity is shown by the very characteristic *Senecio Pseudo-Arnica* (fig. 10), abounding on the strands of Bering Sea, thence southward to Japan and Vancouver, and about the Gulf of St. Lawrence, northward on the coast of Labrador and south very locally to the entrance of the Bay of Fundy.

The illustrations which I have thus far given serve to indicate the chief North American affinities of the flora of New England and the region about the Gulf of St. Lawrence; but these North American affinities are only half the story; for this complex region has in its flora large elements which are identical with or closely related to

floras in the remotest corners of the globe. The best known of these discontinuous floras is, of course, the case of temperate eastern America and temperate eastern Asia, which together share scores of genera and subgenera and even a unique family unknown in other parts of the world, while many more species and geographic varieties are confined to these two most remote regions. This famous group of plants, long ago pointed out by Asa Gray, may be illustrated by the tulip-tree, *Liriodendron Tulipifera* (fig. 11), with two living areas, one from New England to the Great Lakes and the Gulf of Mexico, the other (of var. *chinense*) in China. Very similar ranges are displayed, though often with greater development in Asia, by genera such as the monotypic *Symplocarpus* (fig. 12), by *Magnolia*, *Menispermum*, *Podophyllum*, *Caulophyllum*, *Panax*, *Phryma*, and numerous others. In fact, so frequent is this identity that we are now discovering upon close inspection that common Alleghenian plants, which have long been identified with continental European species, are in reality quite distinct from the European but inseparable from their eastern Asiatic representatives. Thus the common Alleghenian enchanter's nightshade, which for a century and a half has passed as the continental European *Circaea lutetiana*, proves to be not that species but to be identical with the plant of eastern Asia. The disrupted range of this species, *C. latifolia*, is essentially like that of *Symplocarpus foetidus*. Very recently other cases have come to attention. For instance, Butters, in studying certain widely diffused groups of ferns, discovered that in its essential character the common lady fern of eastern America which has generally been considered the European *Athyrium Filix-femina*, is really very different and constitutes a distinct east-American species, *A. angustum*, but that collections from China and Amur show fronds quite inseparable from the east-American plant. Again, in his studies of the variations of *Botrychium virginianum*, Butters found pronounced characters in the sporangia, which separate the European plant as var. *europaeum*, but that the typical *B. virginianum* of temperate eastern America reappears in China.

Other plants of much broader and almost general occurrence throughout temperate Eurasia are found in America only at the extreme eastern margin of the continent. Such a species is *Stellaria uliginosa* of wide Eurasian range, and found locally in springy spots from Newfoundland to Maryland. Of more restricted American range is *Potamogeton polygonifolius*, generally dispersed over Eurasia

and even in Madagascar and New Zealand, found on the Azores, and filling the ponds and streams of southeastern Newfoundland and of Sable Island, 100 miles off the Nova Scotia coast.

More restricted than the latter group is a series of species characteristic of the acid peats and silicious soils of Europe (but not Asia) and in America known only from southeastern Newfoundland or from Cape Breton. There are about 25 of these species, well represented by *Potentilla procumbens* of Europe, Madeira, the Azores, and peaty hillsides and borders of woods in Newfoundland and Cape Breton. A similar distribution is shown by the beautiful pink-flowered *Pedicularis sylvatica*, in America found only in the peaty soils and "heaths" of southeastern Newfoundland where it is accompanied by *Sieglingia decumbens* (fig. 13), a monotypic grass which in the British Isles bears the highly appropriate name "Heath Grass."

Still more obviously the last relics of an ancient broad dispersal are plants now restricted to the extreme western margin of Europe or to the Azores and similarly found only at the extreme eastern margin of North America; such a genus as *Corema* (fig. 14) in the *Empetraceae*, with two known species, one found only in Portugal and adjacent Spain and the Azores, the other from New Jersey to the Gulf of St. Lawrence; or that most distinct of Saxifrages, *Saxifraga Geum* (fig. 15), known only from southwestern Ireland, the Pyrenees and southeastern Newfoundland.

One more European affinity may take our attention for a moment, the maritime plants restricted to northwestern Europe and the Gulf of St. Lawrence region. As illustrations three species may serve: *Atriplex maritima* of the sea-sands from the southern Baltic through the English Channel, and on the sands of eastern New Brunswick, Prince Edward Island and the Magdalen Islands; *Polygonum Raii* of the shores of the British Isles and the Channel, reappearing about the Gulf of St. Lawrence and on Sable Island; and another littoral *Polygonum, P. acadiense*, recently described from Cape Breton and subsequently found to occur as a hitherto undetected species in Europe, where, according to Professor Ostenfeld, it replaces *P. Raii* on the shores of the Baltic and in northern Norway.

These are by no means all the life-areas of the northern hemisphere, but they are sufficient, it will be agreed, to indicate that there are few regions of boreal and temperate North America and Eurasia which do not show identities with or close affinities to the complex flora of

the New England-Gulf of St. Lawrence district. But these striking relationships are not confined to the northern hemisphere. South America, Polynesia, Australia and even Africa all show conspicuous cases of identity or generic affinity. One of the widely dispersed genera of the southern hemisphere is *Schizaea*, a group of fern-like plants with 26 species, 25 of which are almost confined to the southern hemisphere (Australia, New Zealand, Polynesia, South America and South Africa), a few of them crossing the equator in the Tropics. No species is known in the Old World north of Madagascar, the Seychelles, India and the Philippines; *i. e.*, the group is absent from practically the whole continental area of Eurasia and Africa. Similarly in the western hemisphere it is wanting in North America north of tropical Mexico and Cuba, with the single exception of one of the most famous species of the northeastern coastal area. This species, *S. pusilla*, was described by Pursh from the Pine Barrens of New Jersey and almost simultaneously by LaPylaie from Newfoundland, LaPylaie making the discerning observation that the same species had been collected by Gaudichaud on the Falkland Islands. For three fifths of a century the Newfoundland record was held in suspicion, and it was believed that LaPylaie's specimens had really come from New Jersey. In 1879, however, the plant was found in Nova Scotia by Mrs. Britton, and later rediscovered in Newfoundland by Waghorne, and intermediate stations on Cape Breton have been brought to light by Nichols; and I can state from personal observation that the great development of this unique plant is in Newfoundland where, with a species of the coastal plain genus *Bartonia*, it often fills the exsiccated depressions in the tundra. The species is, then, an extreme northern relic of an ancient group now generally confined to the southern hemisphere. I have mentioned LaPylaie's conviction that an identical plant occurs on the Falkland Islands. This is *S. australis*, which certainly is so close to the northern *S. pusilla* that little violence would be done exact classification if they were treated as one.

Of very similar world-distribution is the family *Xyridaceae* but absent from Polynesia and more generally dispersed in Africa. North of Cuba and tropical Mexico the family is found only on the Atlantic slope from Texas to Newfoundland, with a couple of species in peaty habitats about the Great Lakes. The *Haemadoraceae* (fig. 18), likewise, belong primarily in the southern hemisphere, with 17 species in Australia, 11 at the Cape of Good Hope, and the remaining remnant

localized from northern Brazil to Vera Cruz and by way of the Antilles and the coastal plain extending to eastern Massachusetts.

These austral groups, *Schizaea*, the *Xyridaceae* and *Haemadoraceae*, are merely illustrative cases of a large series of families and genera, which in temperate North America are confined to a very restricted region of the Atlantic slope. Other genera, widely dispersed in the southern hemisphere and the tropics but essentially unknown in continental Eurasia, are more generally dispersed in North America. Here belongs the xerophytic genus *Pellaea* of southern and eastern Africa, the Cape Verde Islands, the Azores, India, Flores Island, Australia, Tasmania, New Zealand, various Pacific Islands, the Andes, and mountains of southeastern Brazil; and in North America widely dispersed from Costa Rica to British Columbia, Mackenzie and western New England. As notable as any species is the extremely xerophytic *P. densa*, a unique species, with a known occurrence in Costa Rica, arid mountains from California to southern British Columbia and Idaho, locally in the central Rocky Mountains, the Bruce Peninsula in Ontario, and arid mountain-walls of Megantic and Gaspé Cos., Quebec. Here, then, is a species of a widely dispersed austral genus highly developed in the Sierra Nevada and Cascade Mountains but locally abundant at remote points quite to the eastern margin of the continent. Another illustration inevitably suggested by *Pellaea densa* is that remarkable group of xerophytic ferns constituting a well-marked section or subgenus of *Polystichum*. I refer to *P. mohrioides* and its allies (fig. 17). There are four or five species of this alliance, all plants of the highest degree of localization. *P. mohrioides* and other austral species are known only from the Antarctic Prince Edward Islands, 1,200 miles southeast of the Cape of Good Hope, from the Falkland Islands, Tierra del Fuego, and Patagonia, and as the rarest of isolated species in the Andes. In North America we have two species so close to *P. mohrioides* that some authors have considered them inseparable: *P. Lemmoni*, a famous rare species of the mountains of California, Oregon and Washington; and *P. scopulinum* of similar range, though even rarer, and found with *Pellaea densa* on arid mountain-walls of Gaspé County, Quebec.

This Fuegian affinity is not confined, however, to the extreme xerophytes. It occasionally appears in pronounced hydrophytes. For instance, the plant of wet subsaline shores from the Mississippi Valley to the Pacific which has erroneously passed as *Rumex persicarioides* has been

recently demonstrated to be a unique American representative of the Eurasian *R. maritimus*, differing from the Old World plant in constant characters which led Philippi and Dusén to set it off as *R. maritimus*, var. *fueginus*. Outside its broad range in interior and western North America, var. *fueginus* is known on Tierra del Fuego and on the coast from Rhode Island to the Gulf of St. Lawrence, chiefly on the outer islands which persist as a remnant of the continental shelf: Block Island, Martha's Vineyard, Nantucket, Sable Island and the Magdalen Islands.

Three more illustrations, and I shall have finished this long catalogue. Certain genera, chiefly of the southern hemisphere, are noteworthy because of their restriction there to Australia or Australia and New Zealand and tropical South America and their occurrence north of tropical North America only on the Atlantic slope. Of such genera two examples may serve. *Psilocarya* (fig. 16) occurs in tropical Australia and tropical eastern South America and Cuba, and is represented in continental North America by two extremely local species. The most remarkable of these is *P. scirpoides*, as rare a sedge as we have in our flora, known only from wet sands and peats of southern Massachusetts and Rhode Island, and at similar unique stations near the head of Lake Michigan. As our second illustration may be taken the genus *Erechtites* (fig. 19), highly developed in Australia, New Zealand, eastern and northern South America, Central America, tropical Mexico and the Antilles, and represented in eastern North America by the widely dispersed fire-weed, *E. hieracifolia*. The only other species of temperate North America is a unique plant, *E. megalocarpa*, of the sea-strands of southern Cape Cod, there occurring on one of the most ancient of habitats, the strand of the Atlantic.

In fact the ever-shifting but ecologically uniform and never-changing sea-margin is largely inhabited by an extreme relic flora. This has already been pointed out in case of plants of Bering Sea or the North Pacific, occurring likewise on the strands of the Gulf of St. Lawrence, as well as by such plants as *Polygonum acadiense* on the seashores of Cape Breton and of the lands bordering the Baltic. This persistence on our coast of relics of an ancient wide dispersal in saline habitats is well shown by the remarkable *Junci thelassii*. This unique section of the genus has seven living species, all of saline and subsaline habitats and with a distribution "which indicates that they are remnants of an ancient group. *J. acutus* or one of its varieties occurs in

the Atlantic and Mediterranean regions of Europe and northern Africa, the coasts and steppes of southwestern Asia, the Atlantic Islands (Madeira, the Azores and Bermuda), Cape of Good Hope, the coast of California, southern Brazil, Uruguay, Argentina, Chile and the Islands of Juan Fernandez off the coast of Chile. *J. Cooperi* is known only from saline regions of California and Nevada; *J. Roemerianus* only on the coast from Virginia to Texas; *J. austerus* only from Chile; and *J. Kraussii* only from South Africa; while *J. maritimus* is widely but interruptedly dispersed: on the Atlantic and Mediterranean coasts of Europe, southwestern Asia and northeastern Africa, Cape of Good Hope, the Azores, Bermudas, Brazil, Australia, Tasmania and New Zealand, with its only station on the North American coast on Coney Island, New York." The seventh species occupies an area of only a few square rods in a marsh on the southern margin of Cape Cod and on account of its apparent antiquity has been called *J. pervetus*.

I have now closed my long enumeration of the world-floras to which the New England-Gulf of St. Lawrence flora shows strong affinities. If in the enumeration I have omitted any conspicuous areas it must be recognized that it is impossible in one hour to refer to every corner of the globe. It has often been asserted by our friends to the west of New England that the Autocrat was too ready to admit that "Boston State-house is the hub of the solar system"; but at least they cannot deny that Boston is nearer than other large American cities to the center of the Garden of Eden.

I am often urged by those whose interest in phytogeography does not descend to such minute details as actual species and varieties to "write something about the vegetation of New England. We have had enough about its flora; what we need is an account of the vegetation." To which I am forced to reply that, until we know the species and varieties which constitute the flora, it is premature to enter far into generalizations which depend for their value upon unquestionable premises. And that we are just beginning to know the flora of New England and the region about the Gulf of St. Lawrence should be sufficiently apparent when a summer's botanizing by a single pair of workers in the old states of Maine and Massachusetts results, as did the summer of 1916, in the positive extensions of known ranges of 725 species and the addition to the flora of one state or the other of 64 species, 23 of them new to New England and a full dozen quite new to science.

Only a very limited portion of Puritan New England and old French Canada, Acadia and Newfoundland is yet known to the botanist; and hundreds of unnamed alpine tablelands and cañons yet remain to yield a wealth of endemic and relic species. Only about ten of the hundreds of river-estuaries have been even casually explored and each of these has yielded isolated and often endemic colonies of plants. Our sand plains are just being tapped and there are still areas of thousands of square miles in the Gaspé Peninsula and Newfoundland where no man, either white or red, has yet set foot. But the most available source of discoveries for the future is in the little land-locked or kettle-hole ponds which fleck southern New England, Nova Scotia, the Magdalen Islands and Newfoundland like innumerable bits of mirror scattered over a lawn. There are literally thousands and thousands of these tiny ponds and pools without outlets. Many are on the maps but the majority of them have been thought unworthy either recognition on the government maps or the dignity of a name. Perhaps seventy-five out of the tens of thousands of these small ponds and pools have been visited by botanists and everywhere, whether in Rhode Island, southeastern Massachusetts or in the tundra of Newfoundland, the experience is the same: the number of remarkable species discovered in a given area seems limited only by the number of pools visited. I was recently asked by a famous expert on peat-bogs of the Central States what sedge it is which makes up the peat of southeastern Massachusetts. My answer, that the sedge would differ with the different ponds was hardly what he expected but, with due allowance for occasional repetitions and recombinations, the statement is quite true. One pool may be choked by *Scirpus Torreyi*; the next given over to *Eleocharis Robbinsii;* a few rods beyond another full of *Juncus militaris;* then another filled with *Scirpus subterminalis*, while the next is crowded with a rank growth of *Rynchospora macrostachya*. Such is the everyday experience. But the most baffling feature of these numberless pools and pondholes, a condition discovered only two years ago, is the fact that the borders of many of them are inhabited by two entirely distinct floras. During autumns following a rainy summer the water-table is high and the shore of the pond is a wet peat-bog; during seasons with a long summer drouth the shore is a dry sand-beach. One illustration of this feature will serve. The most visited and best known of these ponds is Winter Pond in Middlesex County, Massachusetts, which for three fourths of a century

has been a never-ending source of surprises. For twelve years I have taken my classes there in October with the hope of showing them *Scirpus Hallii*, isolated by more than 1,000 miles from the nearest station in southern Georgia; *Echinodorus tenellus*, isolated by 260 miles from the next station to the south, in southern New Jersey; and *Eleocharis Engelmanni*, var. *detonsa* and *Ludvigia polycarpa* of prairie-sloughs of the Mississippi basin. But for many years, since 1908, Winter Pond was low and the shore a sandy beach, with the result that these plants have not flourished. In their stead have been found such xerophilous species as *Aristida gracilis*, *Crotalaria sagittalis* and *Cassia nictitans*. In 1916, however, the summer was extremely rainy and when, in October, I took my class to Winter Pond to see the *Crotalaria* and *Cassia*, we found the shore covered with wet peat, with a dense carpet of the long-lost *Scirpus Hallii*, the *Echinodorus*, *Eleocharis* and *Ludvigia* and practically no *Cassia* nor *Crotalaria* to be found. Similar experiences were noted on Cape Cod, and as a result we now understand that we cannot really know the floras of these thousands of pond-shores until they have all been intensively studied in both wet and dry years and throughout the season. When, therefore, the botanist who still retains a New England conscience is urged to "dash off something about the vegetation of New England," he naturally hesitates to write about what he knows he does not yet understand.

Throughout this presentation I have used the term phytogeography, not because that term as often used in America signifies an accurate knowledge of plant-distribution, but because it is a term which ought to stand for a scholarly and precise branch of our science. Unfortunately, many Americans who have styled themselves phytogeographers have not hesitated to stultify the subject by the publication of the point of view that, from the phytogeographer's standpoint, the exact identity of the plants is of little consequence. So long as any "phytogeographers" hold such views they must not expect to win the commendation of those who are striving for final truth. Imagine such sentiments expressed by Linnaeus, Wahlenberg, Alphonse de Candolle, Darwin, Hooker, or Gray! In the American rush to see ourselves in print and not to trouble about precision of detail we are too apt to forget the wise saying of Dr. Holmes: "Knowledge and timber shouldn't be used till they are seasoned." As I have elsewhere had occasion to say, "Much inaccurate and unscholarly publication has seriously injured taxonomy; the same tendency intensified has cheap-

ened ecology; and, unless we take the utmost pains to verify all compilations and to publish only what we have critically studied and digested, we shall soon cheapen and discredit phytogeography as well." Let us then set a high rather than an easy-going and off-hand standard, and phytogeography, which requires the most discriminating knowledge of exact identities as well as a broad outlook upon world-affinities and the power to draw logical deductions, will take in our country the dignified position of authority it has occupied in Europe.

It is frequently said with some suggestion of sarcasm that New England is the region where botanists still carry a vasculum and collect specimens. Yes, it certainly is! And from what I have today outlined in the merest framework of a sketch it is obvious that the New Englander and his Canadian and Newfoundland neighbors will botanize for generations to come before they fully unravel their complicated flora and the vast processes by which vascular plants of nearly all regions of the globe have reached their unique corner of the North American continent.

GRAY HERBARIUM,
HARVARD UNIVERSITY

EXPLANATION OF PLATES XII–XIV

PLATE XII

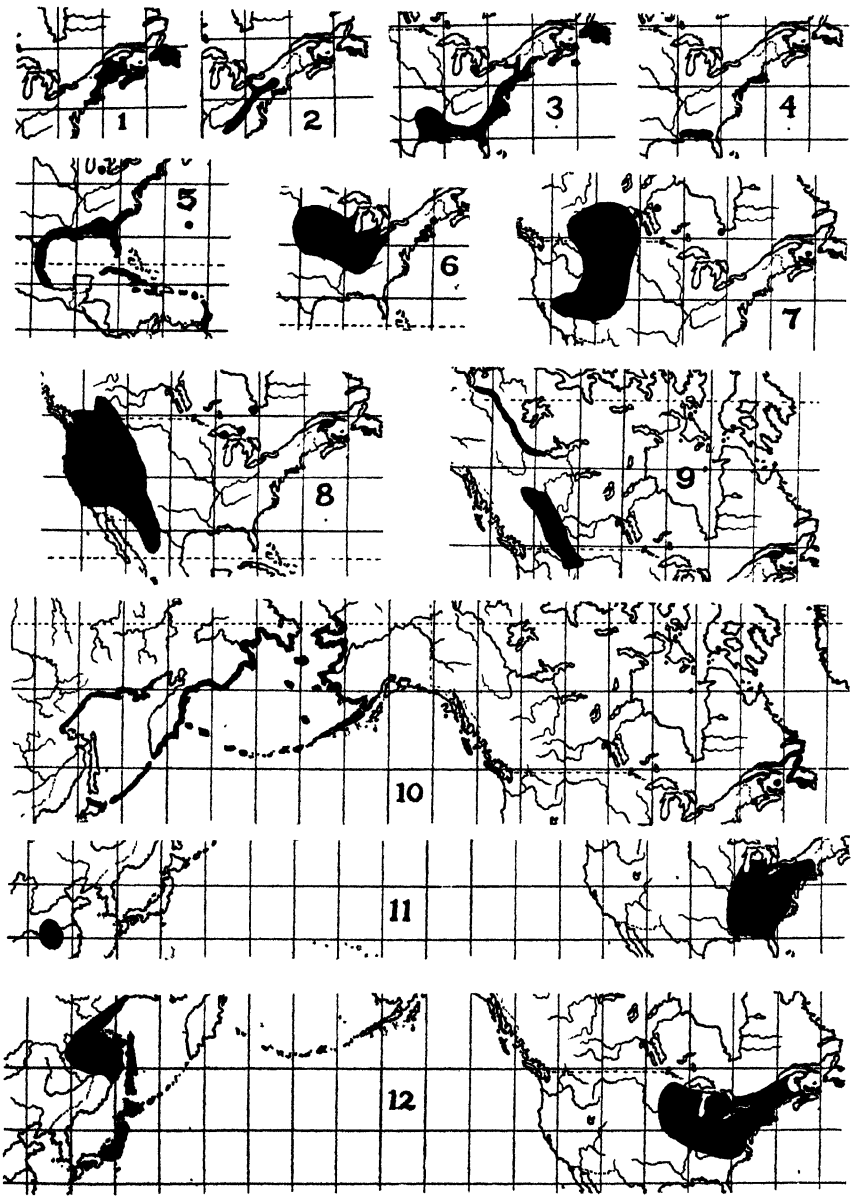
Range of 1. *Rosa nitida;* 2. *Ilex monticola;* 3. Genus *Bartonia;* 4. *Drosera filiformis;* 5. *Eleocharis interstincta;* 6. *Ludvigia polycarpa;* 7. *Potamogeton filiformis,* var. *Macounii;* 8. *Lonicera involucrata;* 9. *Dryas Drummondii;* 10. *Senecio Pseudo-Arnica;* 11. *Liriodendron Tulipifera* and var. *chinense;* 12. *Symplocarpus foetidus.*

PLATE XIII

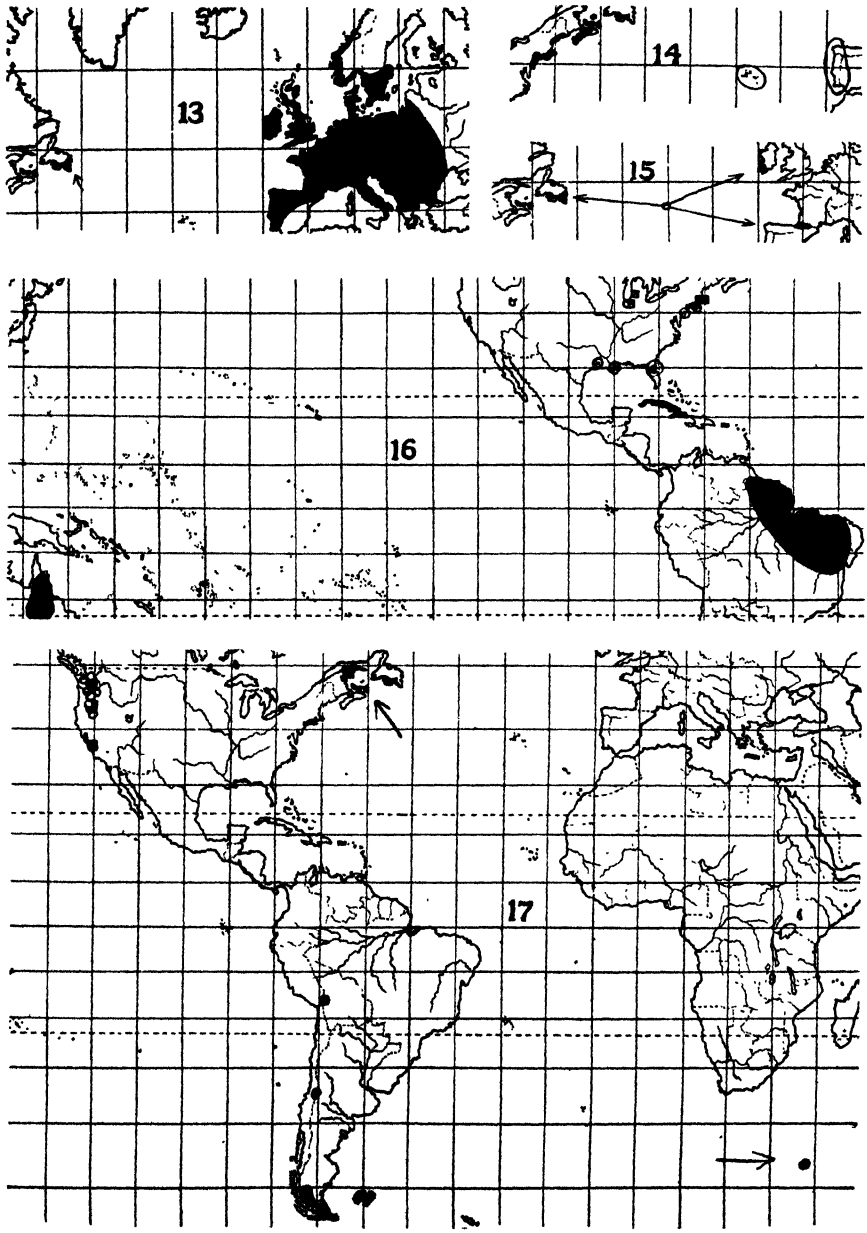
Ranges of 13. *Sieglingia decumbens;* 14. Genus *Corema, C. alba* in outlined ellipses, *C. Conradii* in solid black. 15. *Saxifraga Geum.* 16. Genus *Psilocarya, P. nitens* in circles, *P. scirpoides* in squares, remaining species in solid black. 17. *Polystichum mohrioides* and allies, *P. scopulinum* in solid black squares, *P. Lemmoni* in circles, the remaining species in solid black dots and ellipses.

PLATE XIV

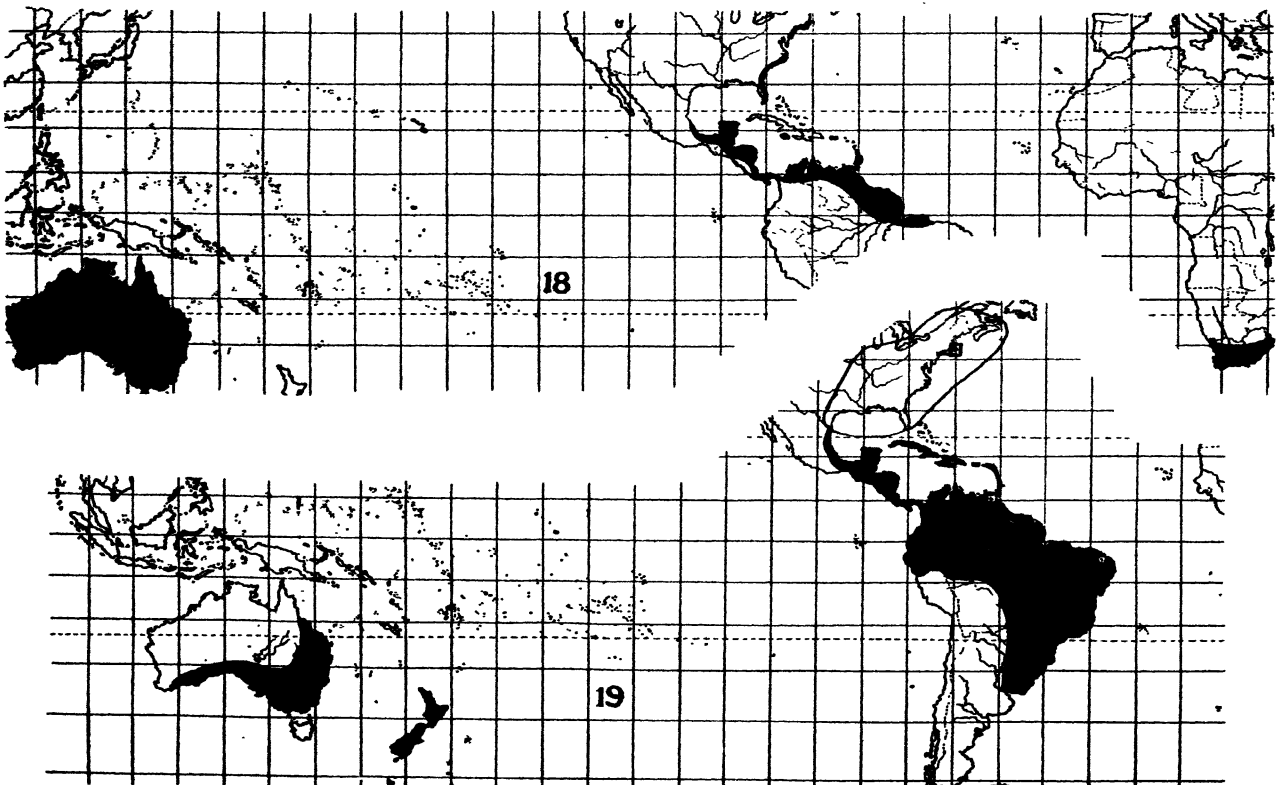
Ranges of 18. Family *Haemadoraceae.* 19. Genus *Erechtites, E. hieracifolia* in the outlined ellipse, *E. megalocarpa* in the small square, the remaining species in solid black.



FERNALD: GEOGRAPHIC AFFINITIES



FERNALD: GEOGRAPHIC AFFINITIES



FERNALD: GEOGRAPHIC AFFINITIES

THE CONTRAST IN THE FLORAS OF EASTERN AND WESTERN NEWFOUNDLAND[1]

M. L. FERNALD

The island of Newfoundland, with an area of more than 42,000 square miles, has a flora as yet only partially worked out, but sufficiently known to indicate a surprising degree of complexity in its makeup. The first impression gained by a casual observer in crossing Newfoundland is that the flora, as one visiting botanist has said, is an attenuated Canadian flora; but further study of the details and a careful daily record of observations on the plants of the island through several seasons of exploration has clearly emphasized that the *attenuation* of the Canadian element is one of the most conspicuous features of the Newfoundland flora. For, although lying in the latitudes of eastern Canada, Newfoundland has an almost negligible strictly Canadian element in its flora. The number of species characteristic of eastern Canada (Nova Scotia, New Brunswick, and the Gaspé Peninsula) and also found upon Newfoundland is made up primarily of such plants as extend their northeastern ranges along the north shore of the St. Lawrence quite to the Straits of Belle Isle and have obviously reached Newfoundland by crossing the narrow Straits.

Besides this meager Canadian flora which has been derived chiefly by way of its northeastern extension to the Straits of Belle Isle, the essential elements of the Newfoundland flora are three: (1) the arctic-alpine and Hudsonian elements, of somewhat broad distribution in the arctic regions or in Labrador; (2) the coastal plain element, a group of species abundant to the southwest of Newfoundland, chiefly in coastwise New England, Long Island and New Jersey; (3) the Atlantic European element, species characteristic of the region from the Baltic or the English Channel to the Mediterranean; while the endemic species and varieties are all closely related to members of the other four groups and should, in point of origin, be classed with them. As I have elsewhere shown,[2] the essential absence of the plants one

[1] Presented at the joint session of the Botanical Society of America and the Ecological Society of America at Pittsburgh, 1 January, 1918.

[2] Rhodora, 13: 141, 142. 1911.

would expect in Newfoundland, that is, the typical plants of the same latitude in eastern Canada, such species for instance as *Clematis virginiana*, *Asclepias syriaca*, *Populus grandidentata*, *Acer pensylvanicum* and *A. Saccharum*, *Eupatorium perfoliatum*, *Solidago squarrosa*, *Solidago juncea*, *Aster macrophyllus*, *Aster acuminatus*, etc., indicates that the flora of Newfoundland, except such species as have been derived across the narrow Straits of Belle Isle, has not reached the island by ocean currents or by winds, especially from the west and southwest; for, if these factors were important in carrying the western and southwestern plants to Newfoundland, we should expect such wind-distributed species as I have named and which are all abundant at the eastern edge of Canada to have reached Newfoundland amongst the first invaders.

A similar absence of the ordinary Canadian mammals and resident birds is conspicuous; for example, the common moose, red deer, porcupine, and spruce partridge, of all the Canadian forests opposite, are quite unknown in Newfoundland, and there the mammal- and resident bird-fauna is composed, like the flora, of species derived from Labrador or from the southwestern coastal margin of the continent, while certain land-snails have been pointed out as identical between Newfoundland and western Europe. In other words, the animal life of Newfoundland shows the same derivation as the plant life.

In explaining[3] the migration to Newfoundland of a large element from the Atlantic coastal plain of the United States it has been necessary to reconstruct the Tertiary continental shelf, which is now depressed as a shallow bench off the east Atlantic coast of America; and from the botanical and zoological evidence, as well as from recently published geological evidence,[4] it now seems perfectly settled that the continental shelf formed in the late Pleistocene and even later a nearly continuous although somewhat interrupted floor from New Jersey and southern New England, by way of Sable Island and the Grand Banks, to southern and eastern Newfoundland. And upon this floor the southern flora and fauna migrated to Newfoundland; but the unfavorable conditions of a sand-floor with meager forest and coastal plain bogs and barrens proved unattractive to the life of our rich Canadian forest, with the result that the forest species both of animals and plants, or the species which demand rich or basic soils, were for the most part unable to cross to Newfoundland.

[3] Rhodora, 13: 135–162. 1911.

[4] Barrell, Amer. Journ. Sci. IV. 40: 1–22. 1915.

The Atlantic European element in the flora would seem to be a relic from the early Tertiary flora which occupied the then dry northern floor of the Atlantic and which had persisted as a small remnant upon the Tertiary continental shelf and at the final submergence of the shelf became stranded upon Newfoundland, which, as is well known, is bordered by a tremendous shelf, the Grand Banks. The arctic-alpine and Hudsonian elements, as already intimated, have presumably entered Newfoundland in post-Pleistocene times by way of the narrow Straits of Belle Isle, which are commonly closed during the winter, thus forming a perfectly simple bridge from the north side of the Straits to the Newfoundland shore.

It is not, however, my intention to develop in this brief paper a theory in regard to the origin of the Newfoundland flora. The chief points I wish to emphasize are certain features which are of more practical and immediate interest to a group of ecologists. The most striking physiographical features of Newfoundland, so far as they impress the visiting botanist, may be very briefly summarized as follows.[5] Extending from the southwest corner of the island at Cape Ray eastward for several miles, thence as a broad belt northward along the west coast to within 20 miles of the Straits of Belle Isle, is the Long Range of mountains. These for the most part are high tablelands of very diverse rock structure, the western tablelands and valleys and the broad foreland (20 miles wide at the north) being highly calcareous, consisting chiefly of limestones, marbles, calcareous slates, calcareous conglomerates, and in some areas of dolomite, traps and serpentines. The eastern ridges of the Long Range are chiefly granitic and they pass on their eastern flanks directly into a great central basin or low tableland of Archaean and chiefly acid rocks. This area, the Great Barrens or central tundra region of Newfoundland, extends, as observed from the train, for a distance of perhaps 100 miles west and east from the eastern flanks of the Long Range to the lower Exploits Valley. From the lower Exploits eastward and southeastward the region becomes again rolling, but without any conspicuous mountains, except a few isolated granitic masses. In this southeastern region of the main island the rocks are essentially all acidic or highly silicious, so much so that the giant pulp and paper mills of the Harmsworth syndicate, located upon the lower Exploits

[5] The most available brief account of Newfoundland physiography is a paper by Twenhofel in Amer. Journ. Sci. IV. 33: 1–24. 1912.

and about Notre Dame Bay, are forced to import all the limestone used in their mills from the west coast. The southeastern peninsula of Newfoundland, the Avalon Peninsula, separated from the main island by an extremely narrow and low isthmus which now consists chiefly of a flat peat bog, is, like the adjacent main island, composed essentially of silicious and acidic rocks. In the extreme southwest also, the region from Cape Ray to Bay St. George, the rocks are chiefly Carboniferous sandstones with little or no calcareous matter, or with such areas small and scattered, and covered extensively with acid peats. Projecting far to the north of the main island and bordered on the northwest and north by a broad foreland of horizontal limestones quite to the Straits of Belle Isle is the North Peninsula or Petit Nord; its interior practically unknown, but its western, northwestern and eastern tablelands almost exclusively of calcareous rock. Such, roughly, are the parts of Newfoundland as yet known to botanists, four distinct areas: the calcareous western region north of Bay St. George, and the North Peninsula; the acid central tundra region; the acid southeastern; and the acid southwestern sections.

So strikingly different are these areas in the composition of their flora that it is difficult to enumerate more than a few score of species which are generally distributed over the island. To the botanist who has spent a season exploring along the west coast, where the soils are calcareous and extremely fertile and the valleys sheltered and sunny, a transfer of base for another season to the southeast is like entering another world. The conspicuous elements in the flora of the west coast are the plants which we have come through long experience to associate with highly calcareous soils, while only upon such acid areas as the Carboniferous sandstones from Cape Ray to Bay St. George, the raw humus of mountain crests or peat bogs, or the granitic mountains at the eastern edge of the Long Range, do we find the plants commonly recognized as inhabiting acid or silicious areas. The valleys of the west coast have long been recognized as the most promising regions of the island for agriculture and adventurous and far-seeing young men from England and Ireland have undertaken extensive agricultural enterprises in the West; and during the past season the successful raising of wheat and the erection of a grist-mill in this region have been heralded by the Associated Press as epoch-making achievements. Contrasted with this favorable condition for agriculture, which prevails through the valleys and the lower levels of the

west coast, a region where fog and bleak winds are dispelled by the warm sun, is the condition in southeastern Newfoundland from Notre Dame Bay to the Avalon Peninsula. This eastern and southeastern coast is vastly more populous than the west coast, the people being chiefly fishermen and miners, but agricultural pursuits are almost negligible in this area. The Arctic Current, after following the Labrador coast, sweeps the east side of Newfoundland as a positive stream clogged into mid-summer with floe-ice and often closing the harbors to navigation; and experiences through several summers, reinforced by the statements of permanent residents, justify the statement that almost any day through the summer season one may look from the eastern shores with the prospect of detecting an iceberg. In other words, the east coast as contrasted with the west coast is bleak, foggy and with a subarctic climate; and the people of the east coast are severely handicapped even in raising potatoes and cabbages.

Now, turning to the vegetation of these two extreme areas, regions separated by 100 miles or more of tundra, we find that in the West the plants of the limestone valleys, talus slopes, brook ravines, river valleys, and open ledges are almost universally species of high northern distribution, occurring in western Newfoundland as outlyers from a broad circumpolar range. Peaty or wet limy depressions of the west coast, for instance, are occupied by *Kobresia caricina*, a characteristic sedge of high-northern distribution, very rare in America except on the north side of the Straits of Belle Isle[6] and in western Newfoundland, or with it *Juncus triglumis* or *Tofieldia palustris*, species which we rarely, if ever, see south of western Newfoundland. With these plants or on wet limestone slopes the calcicolous Saxifrages abound, *Saxifraga oppositifolia* (fig. 1), of the widest circumpolar distribution, extending southward on wet calcareous slopes to western Newfoundland, Anticosti, the Gaspé Peninsula, and the northern Green Mountains, and pushing south into the Canadian Rocky Mountains; or with these species *Saxifraga aizoides*, a characteristic plant of the Canadian Rockies, or *S. caespitosa*, of broad circumpolar range. Rocky ravines and shores of the western coast and the North Peninsula are made beautiful by that handsomest of willows, *Salix vestita* (fig. 2),

[6] Contrary to the general impression that Labrador is a vast barren of Archaean gneiss, it should be pointed out that at the extreme Southeast, along the Straits of Belle Isle, the rocks are Cambrian limestones and sandstones; while the extreme Northeast consists of highly basic ranges of mountains.

a species unknown south of Newfoundland and the Gaspé Peninsula in eastern America, but very characteristic of the calcareous Canadian Rocky Mountains; while the driest of limestone shingle may be carpeted by the arctic-alpine *Salix reticulata*, a close relative of *Salix vestita*, but differing from it in various technical points. Similarly, on the calcareous shingle one is sure to find the handsome *Dryas integrifolia* (fig. 3), again of extensive distribution in the arctic archipelago and other regions of arctic America, but rare so far south as western Newfoundland; or *Potentilla nivea*, of more general arctic distribution and extending south into the Rocky Mountains and in the East as far as the coasts of western Newfoundland and the Gaspé Peninsula. With these plants the very striking *Lesquerella arctica* (fig. 4) abounds on the limestone shingle, again a plant of arctic range, found southward only in northeastern Labrador, on Anticosti Island, and in western Newfoundland; while these limestone plains and tablelands are the home of arctic-alpine Antennarias, Arnicas, Astragali, and various species of *Hedysarum*, *Gentiana*, *Campanula*, *Draba*, *Arenaria* and numerous calcicolous ferns. In the wetter valleys *Parnassia Kotzebuei*, of broad arctic distribution and local occurrence in the Canadian Rocky Mountains, is found and with it such characteristic Rocky Mountain plants as *Juncus longistylis*, *Cryptogramma Stelleri*, *Poa alpina*, *Cypripedium parviflorum*, and *Viola nephrophylla*, while occasionally a wet bank will be encountered covered with a dense carpet of the extremely arctic *Carex glacialis*, unknown elsewhere in America south of the arctic realm. These characteristic plants of western Newfoundland, then, are the species of high arctic-alpine range, abounding in America chiefly in the arctic archipelago or in the Canadian Rocky Mountains, both areas composed almost entirely of calcareous rock. This distinctive flora, which gives character to the west coast, consists of some hundreds of species which are quite unknown from the east coast or from the central tundra district.

When we come to the east coast the first impression of every traveler is one of excessive barrenness and untempered bleakness. In this region of acidic rocks the rich forests of the valleys of the west coast are not met. The trees are small and chiefly stunted, and plants which give the pronounced character to this region of subarctic aspect are very different from those of the west coast. On the peaty slopes of the hills of southeastern Newfoundland one finds himself divided in his mind as to whether the flora is more like that of the heaths of

England and western France or of the barrens of New Jersey, for here is a peculiar mingling of plants characteristic of the peats and silicious soils of Atlantic Europe and of the northern coastal plain of the United States. The peaty tracts are brilliant in August with the delicate, pearly-pink flowers of *Pedicularis sylvatica*, one of the most characteristic species of humus in western Europe, known nowhere in America except in southeastern Newfoundland, where it is accompanied in the peaty and heathy slopes not only by the heather itself, *Calluna vulgaris*, but by a unique grass, *Sieglingia decumbens*, a species of the peaty and heathy soils of Europe which in Great Britain bears the significant name "Heath Grass"; and one will find in southeastern Newfoundland with these characteristic European oxylophytes another of their European associates, the strong perennial *Potentilla procumbens*, resembling our *Potentilla canadensis*, but with many technical differences. This species, characteristic of western Europe, Madeira, and the Azores, is unknown in America except in the peaty slopes and wood-borders of southeastern Newfoundland and Cape Breton, although there is a vague early report of its having been collected in southern Labrador. Several other Atlantic European plants, altogether about 25 species, quite unknown in America outside eastern Newfoundland or occasionally Cape Breton or Sable Island, 100 miles off Nova Scotia, might be enumerated, but the species already mentioned are sufficient to indicate the Atlantic European element in the peaty soils of the region.

Associated with these plants one will find *Solidago uniligulata*, a characteristic plant of the New Jersey pine barrens; *Gaylussacia dumosa*, the coastal plain huckleberry, extending from the Gulf of Mexico around the entire coastal plain of eastern America; the inevitable cranberry, *Vaccinium macrocarpon*, which in the Yankee mind immediately suggests Cape Cod or New Jersey; or *Aster nemoralis*, a characteristic plant of southern New England and New Jersey. Turning to the more favorable habitats, the river-silts and -gravels, one will find likewise the strong European affinity in such species as *Juncus bulbosus*, unknown in America except in eastern Newfoundland and on Sable Island, while the coastal plain affinity is conspicuous in such plants as *Sisyrinchium gramineum*, abundant throughout the southeastern United States, becoming rare north of Massachusetts and quite unknown east of central Maine except as localized on Sable Island, the tip of Gaspé, and in southeastern Newfoundland; or

Panicum tennesseense, one of the most abundant grasses of the southern and southeastern United States, abounding eastward as far as Maine and western New Brunswick, but unknown from that region eastward until we come to the extreme eastern edge of Newfoundland. Turning now to the aquatics of eastern Newfoundland, these show exactly the same peculiar geographical ranges. The ponds and lakes of eastern Newfoundland are given over to such species as *Potamogeton polygonifolius*, the common pondweed of European heath-lands, of broad Eurasian distribution, but quite unknown in America except in southeastern Newfoundland and on Sable Island, or *Potamogeton Oakesianus*, the commonest pondweed of Nantucket and Cape Cod and found also southward into New Jersey.

So much, briefly, for the characteristic flora of the peaty open woods and slopes, the river-banks and ponds. The more exposed rocks and sand hills also show a strong coastal plain affinity. Everyone familiar with the open pine woods and sand hills of Atlantic United States knows the genus *Hudsonia*, represented by the two species, *H. tomentosa* and *H. ericoides*. Both of these species reach Newfoundland, but the latter, *H. ericoides* (fig. 5), is notable because it is found only in the extreme eastern portion of the island, where, like many other southern plants, it occurs on islands surrounded by nearly perpetual ice or ice-floe and fog. Thus, briefly, we have summarized the main floral elements of the characteristic acid southeastern region of Newfoundland.

Turning now to the central tundra district, we find, on referring to standard literature upon phytogeography, that the interior of Newfoundland is called a part of the arctic tundra; but this, like much of recent phytogeographic literature, is a statement prepared far away from and with a minimum of knowledge of the region described, for, although the region is certainly tundra, the most conspicuous thing about the tundra is the fact that it is not arctic. One of the larger ponds at the eastern edge of the tundra district bears the name, at once attractive to the visiting botanist, Rushy Pond. When our party was in this region, one of the first guesses was, inevitably, as to the particular rush which gave name to the pond. But after our experiences already in the region we all guessed alike, that it must be the common coastal plain *Juncus militaris*. The guess was correct and Rushy Pond was found to be bordered, like the ponds of New Jersey, Long Island and Cape Cod, by *Juncus militaris* (fig. 6), while

floating amongst the *Juncus* culms were the characteristic leaves and delicate flowers of *Nymphoides lacunosum*, with *Potamogeton dimorphus* and other southern species abundant. Going into the most characteristic tundra itself, for instance the vast tundra-region of bog and shallow pools near the station Quarry, one finds the arctic plants disappointingly few and only such species as are equally Hudsonian and Canadian in range. Pond-holes here, in the most highly developed tundra, are again full of *Juncus militaris*, or *Carex exilis*, *C. livida* or *Scirpus subterminalis* of the New Jersey pine barrens, while the pine barren *Potamogeton confervoides* fills the pools and the bushy patches are bordered by *Carex folliculata*, a species extending to Florida and Louisiana. In autumn, after the long August drouth, the little pools of the tundra have mostly dried away leaving peaty depressions which are carpeted with *Lycopodium inundatum*, *Eriocaulon septangulare*, *Bartonia iodandra*, the Newfoundland representative of the coastal plain genus *Bartonia*, *Schizaea pusilla*, the famous curly grass of the New Jersey pine barrens, *Xyris montana*, the northern outlyer of the austral and coastal plain genus *Xyris*, and many other southern species, the enumeration of which would become wearisome.

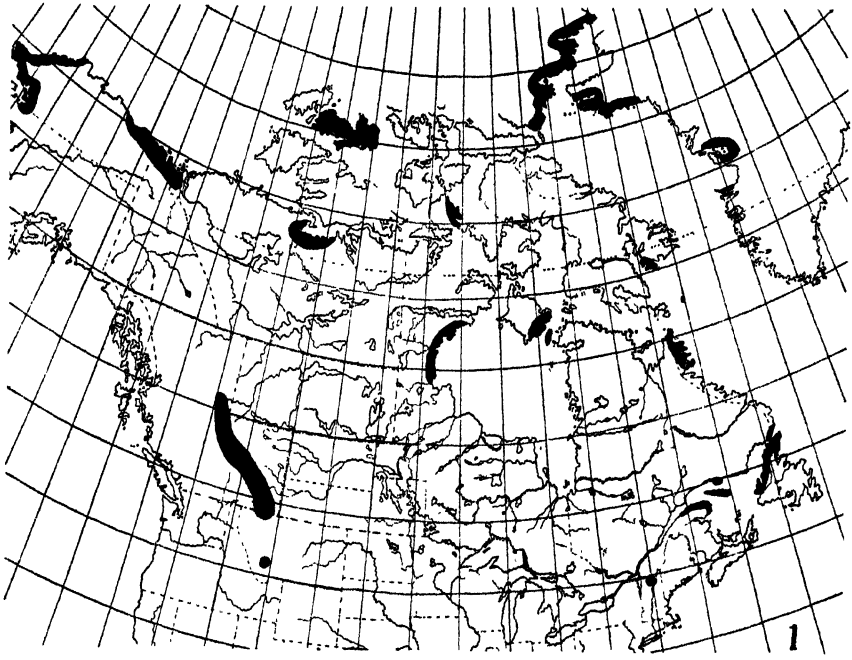
Similarly in southwestern Newfoundland, in the Carboniferous sandstones about Bay St. George, is a flora which is decidedly austral and disappointing to one who goes to the region looking for boreal plants: tremendous sphagnous bogs with an abundance of *Arethusa*, *Calopogon*, *Habenaria blephariglottis* or *Carex exilis*, just as if one were botanizing in New Jersey, while the drier areas furnish *Melampyrum lineare*, *Carex intumescens*, *Salix humilis*, *Diervilla Lonicera*, *Populus tremuloides*, and others making a tedious and uninteresting flora.

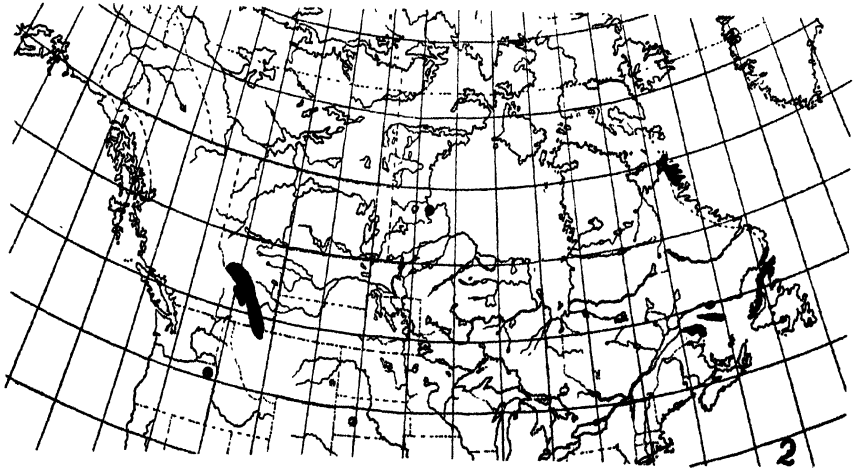
Now from this statement, which is a very brief summary of the conditions in the flora of Newfoundland, it must be apparent that the highly silicious or acid areas, such as the extreme eastern region of Newfoundland, the central tundra district, and the southwestern corner, are populated chiefly by plants of coastal plain origin with an admixture of species belonging primarily in the acid soils of Europe, which reached the island by way of the continental shelf; while the calcareous west coast and North Peninsula is characterized by a flora which finds its great development here and in the calcareous arctic archipelago and the calcareous Canadian Rocky Mountains. Yet the west coast with its arctic flora is the warm, sunny, and most fertile region of the island, while the east coast is the cold, bleak, and more

barren district. It is sufficiently evident to "him who runs" that the southern coastal plain plants, including such extreme austral genera as *Schizaea*, *Bartonia*, and *Xyris*, are in the acid regions of Newfoundland not because these regions are subarctic in climate but because the plants there find the acid soils which abound in the coastal plain region of the southeastern United States where these genera also occur. It is equally patent that the calcicolous arctic species which abound on the warm west coast of the island are there not because that is the warmest and most temperate region of the island, but because they there find the calcareous soils which are essentially like those in the other areas where they abound.

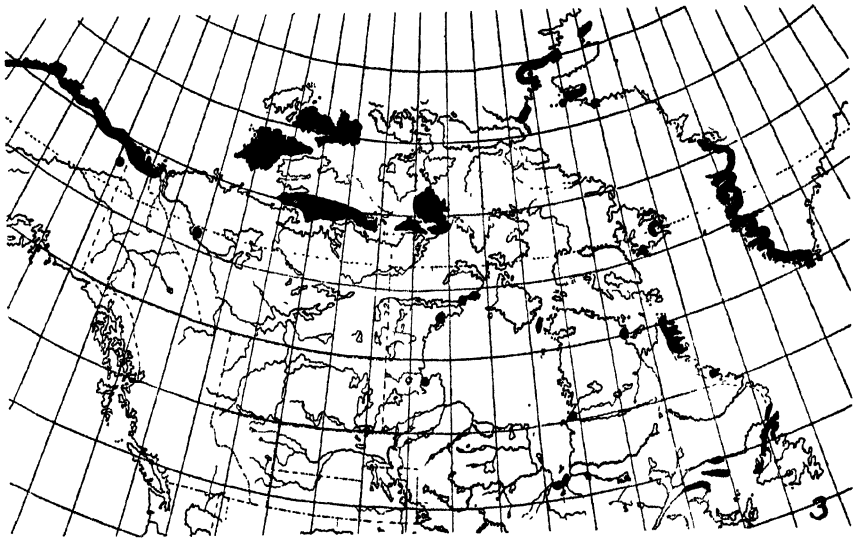
Now, as a corollary of this analysis one very striking feature comes out. This is the complete absence from acid central and southeastern Newfoundland of many Hudsonian and arctic-alpine species of acid Labrador and the granitic mountains of eastern Quebec, New England, and northern New York, species which are so general upon the mountains of New Hampshire and Maine and in the acid Labrador region that one would inevitably assume that they must abound in Newfoundland. Nevertheless, two centuries of botanizing in Newfoundland by hundreds of good botanists, ranging in acumen from Sir Joseph Banks and Bachelot de la Pylaie to the most humble amateur, has failed to reveal in Newfoundland such widely spread oxylophytes as *Arenaria groenlandica* (fig. 7), the commonest of plants on all granitic mountains of New England, as well as a widely dispersed plant of Labrador and Greenland; *Viola palustris*, which borders the mountain brooks of New England, the granitic Table-top Mountain of Gaspé, Labrador and the general northern regions; *Salix herbacea* (fig. 8), the little willow which carpets the wet humus of New England and Labrador mountains; *Cardamine bellidifolia*, abundant in sheltered pockets of granitic rocks of New Hampshire, Maine, Table-top Mountain, and acid northern regions; and so on through a long, long list. A few species such as *Poa laxa*, *Hierochloe alpina*, *Luzula spicata*, *Salix argyrocarpa*, *Betula glandulosa*, *Phyllodoce coerulea*, and *Cassiope hypnoides*, which are very abundant in all our granitic mountain regions of New England, Labrador, and the far North, have been found at one or, in rare cases, two isolated stations in Newfoundland. But it is obvious that they are rare and have just made their debut on the island.

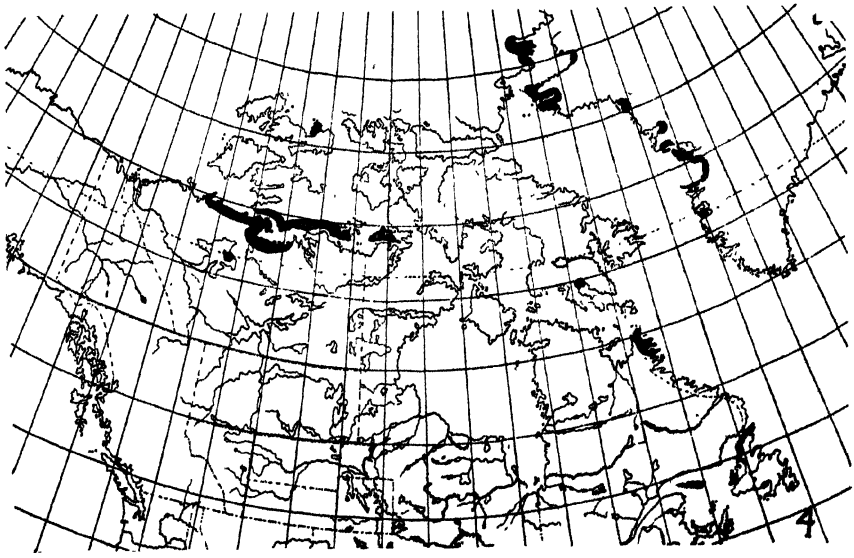
In connection with this extreme paucity of the oxylophytic arctic-

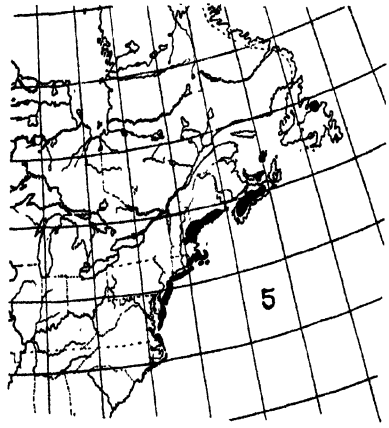


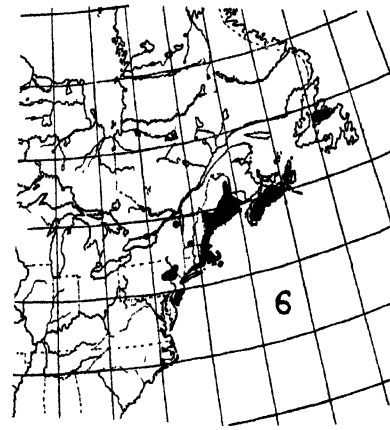


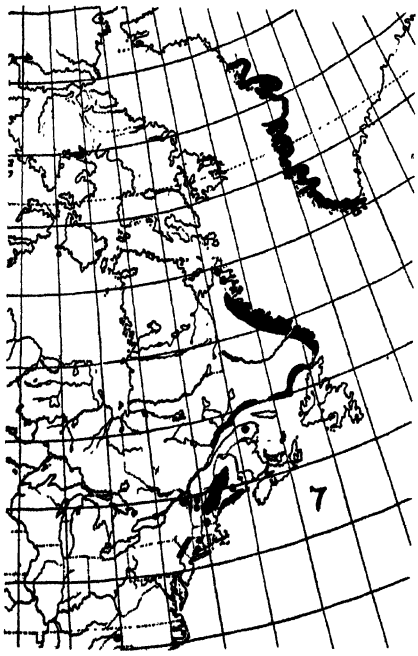
FERNALD: FLORA OF NEWFOUNDLAND

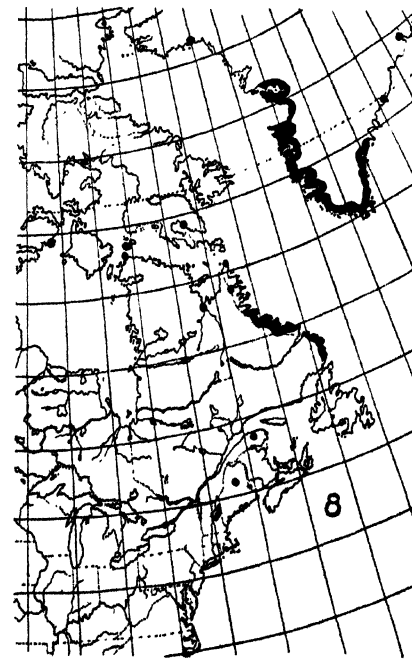

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alpine flora in the acid, bleak, subalpine central and southern regions of Newfoundland, it is noteworthy that the North Pensinula, which separates the vast acid area of Newfoundland by scores of miles from eastern Labrador, is itself essentially a limestone region. It thus would seem that, although these oxylophytes abound on adjacent Labrador, their first landing, when they are blown as seeds or fragments across the Straits of Belle Isle to the Newfoundland coast, would be upon a forbidding limestone soil. Consequently the majority of these species have not yet achieved a successful start, although they are doubtless blown to the Newfoundland coast many times during every winter. In a few cases seeds have succeeded in passing the limestone barrier and colonies are now starting as new occupants of the acid region.

From this brief analysis of the components of the Newfoundland flora it should be apparent, I think, that, if we are to get at the fundamental ecological laws, we must take more thoroughly into account than is generally done the elementary principle that many, if not most, plants are highly selective in their soil requirements. Explain away this point as we may, it constantly obtrudes itself, and it is certainly the part of wisdom to recognize facts as they are and to take as a working principle the general formula, that *the presence or absence of varying degrees of available lime or of other bases in the soil is more fundamental in determining plant distribution than are even considerable differences of temperature and humidity.*

GRAY HERBARIUM,
HARVARD UNIVERSITY

EXPLANATION OF PLATES XV–XVII

PLATE XV

1. American range of *Saxifraga oppositifolia.*
2. Range of *Salix vestita* (including varieties).

PLATE XVI

3. Range of *Dryas integrifolia.*
4. Range of *Lesquerella arctica* (including var. *Purshii*).

PLATE XVII

5. Range of *Hudsonia ericoides.*
6. Range of *Juncus militaris.*
7. Range of *Arenaria groenlandica.*
8. American range of *Salix herbacea.*

GENERIC TYPES WITH SPECIAL REFERENCE TO THE GRASSES OF THE UNITED STATES

A. S. Hitchcock

Efforts have been made in recent years to stabilize nomenclature by proposing rules to govern nomenclatorial changes. An important advance in the progress of nomenclatorial reform was made when the idea of types was introduced, the idea that a genus should be based upon a type species, and that a species should be based upon a type specimen. In the future an element of stability will be introduced if authors of generic and specific names will definitely designate the types of the groups they publish, something rarely done except within recent years. If the idea of types is introduced into our nomenclatorial system, and if the application of the idea is made retroactive, it becomes necessary to select types for groups for which no type was designated by the author.

The present paper is concerned with generic types. Rules have been proposed by committees and congresses for the selection of type species of genera. The intention has been so to frame these rules that they may be applied automatically, that all investigators shall arrive at the same result in applying them, and that individual judgment shall be eliminated. However, it has been impossible to foresee all contingencies, and experience has shown that no such set of rules can be automatically applied with satisfactory results. I doubt if rules can be so framed as to eliminate personal judgment, and I furthermore deprecate such an attempt. I believe that an effort should be made to agree upon principles and that judgment should be used by the individual in making application of the principles to individual cases. I furthermore believe that the road to uniformity lies through agreement rather than through the arbitrary application of rules. From whatever standpoint the question of generic types is viewed, it is evident that proposed action to obtain uniformity should be based upon a knowledge of the facts concerning a fairly large number of cases.

As a basis for a solution of the question the generic names of the grasses of the United States have been investigated, the facts bearing

upon the selection of types have been separated and arranged, and the type species selected according to certain general principles that will be set forth in the present paper. Types have been selected for proposed genera even though those genera may not be accepted as valid, because a non-valid name is referred as a synonym according to the identity of its type species.

Certain definitions and principles are the basis of the work here presented.

The type species of a genus determines the application of the generic name.

In any combination or division of groups the genus, however limited, must include the type species.

The type species is the species or one of the species which the author had chiefly in mind when the genus was established. We may often be justified in assuming that a certain species is the basis for a generic idea because of the fact that the author has figured this one or by the fact that he actually examined, or was more familiar with, a particular species. Sometimes a careful reading of the generic description makes it evident that the author based this description upon a particular species even though more than one species was included in the genus.

A change of name or a substitution of one name for another does not change the type.

The type is determined upon the basis of facts given with the original publication of the generic name. These facts may sometimes be interpreted by previous or subsequent historical data.

In a large number of cases it is easy to determine the type species directly, with results acceptable to all. There are, however, a considerable number of cases in which a more or less arbitrary selection must be made and in which the judgments of competent persons will differ as to the species selected. In the present paper space permits only a summary of results.

The generic names investigated number 255. These may be classified as follows:

1. The type species has been designated. Total 8.
2. Type not designated.

a. Monotypic genera, those in which only one species was mentioned at the time of the original publication. Total 150. In these monotypic genera the single species is indicated in a variety of ways.

(1) The species may be described, either as new, or as a transfer from another genus.
(2) The species may be mentioned without description under a described genus, as is frequently the case when a new genus is based upon an old species.
(3) A new generic name may be applied to a species previously described.
(4) The new genus may be connected with a previously published species by an indirect citation. Most of Adanson's genera are published in this way. Under the name Valota appears the citation of a plate in Sloane's History of Jamaica. Linnaeus cites the same plate under *Andropogon insularis*. Hence Valota Adans. is based on *Andropogon insularis* L.

b. More than one species mentioned with the original description. In these cases a selection must be made. The principle underlying the selection is to choose the species that seems most nearly to represent the author's concept of the genus. We may usually assume that a figured species represents this concept, as an author naturally picks out for illustration a typical species. Therefore, in general, a figured species is selected as the type. If more than one species is figured, the type is assumed to be one of the figured species. Sometimes certain species can be excluded from consideration as the type because they are referred somewhat doubtfully to a new genus by the author or because they do not agree perfectly with the generic description. From those available one may often assume, as most typical, a well-known economic species, or the historically oldest, or one native in the author's country or familiar to him in cultivation. If there are two or more species equally available as the type and one must be chosen arbitrarily, then we may well choose the one which results in the application of the generic name in the commonly accepted sense. Usually the choice of the first of the equally available species accomplishes this result. In order to illustrate the manner in which type selection works out in practice, several illustrative examples are given below.

Coix L. Sp. Pl. 972. 1753. Linnaeus describes 2 species, *C. lachryma-jobi* and *C. dactyloides*. In typifying the genera of the Species Plantarum, it is necessary to consider at the same time the fifth

edition of Linnaeus's Genera Plantarum which appeared the following year. There are no descriptions of genera in the former work, these being set forth in the latter. Linnaeus often cites, in that place, a figure in some earlier work which may determine the type. Under Coix is cited Tournefort's plate 302, which represents the first of the two species above mentioned. We are thus justified in selecting this species as the type of Coix.

ERIANTHUS Michx. Fl. Bor. Amer. 1: 54. 1803. Michaux describes 2 species, *E. saccharoides* and *E. brevibarbis*. He derives the generic name from two Greek words meaning hairy flower because the flowers are involucrate with very dense wool. The first species is selected as the type because the spikelets are very woolly, while in the second species the hairs are short.

ANDROPOGON L. Sp. Pl. 1045. 1753. Linnaeus describes 12 species. The reference in the Genera Plantarum is "Roy. lugdb. 52," that is, the Flora Leydensis, published in 1740, in which Royen, the author, describes 2 species of Andropogon. I think the type should be selected from these two. There is no reason to think that one of these was more familiar than the other to Linnaeus or to Royen. *Andropogon virginicus* is selected as the type because this has priority of position in the Species Plantarum, and because this selection retains the generic name for the group universally known as Andropogon. The other species, *A. hirtus*, belongs to the genus or subgenus Cymbopogon. If *A. hirtus* were made the type of Andropogon, that name would have to be applied to the group now known as Cymbopogon and the genus long known as Andropogon would have to receive a different name. Logical typification may lead to confusing shifting of names, but confusion should not be brought about by the arbitrary selection of the type species.

HOLCUS L. Sp. Pl. 1047. 1753. Seven species are described by Linnaeus, *H. sorghum*, the nonsaccharine sorghum, *H. saccharatus*, the sweet sorghum, *H. halepensis*, the Johnson grass, *H. lanatus*, the velvet grass, and three other little-related species. The nomenclatorial history of these species shows a conflict between concept and fact, between what should have been done and what was done. The first 3 species were segregated from the others in 1763 by Adanson who applied to them the old pre-Linnaean name Sorghum. The last three of the original 7 species were assigned to other genera, leaving under Holcus the remaining species, *H. lanatus*. This procedure was equiva-

lent to the selection of *H. lanatus* as the type of Holcus. What should have been done, and what is herewith done, was to select *H. sorghum* as the type species, for the following reasons: In the Genera Plantarum Linnaeus cites, under Holcus, the name "Sorgum Mich.," indicating that he was applying the name Holcus to what was called Sorgum by Micheli and others of his time, that is, to what we call Sorghum. Furthermore and most important, the description of the genus Holcus in the Genera Plantarum applies only to the sorghums and not to the other 4 species in the Species Plantarum. Therefore I have selected *Holcus sorghum* as the type of Holcus. Holcus then becomes the equivalent of Sorghum and replaces that as a generic name. This is one of the few cases where a logical selection of the type species changes the application of a well-known name among economic plants.

LEERSIA Swartz, Prod. Veg. Ind. Occ. 21. 1788. Swartz describes 3 species. All are equally available as type species. The third is chosen because it is the oldest historically, being based on *Phalaris oryzoides* L., the other two being described by Swartz as new.

PHALARIS L. Sp. Pl. 44. 1753. Five species are described by Linnaeus. The first, *P. canariensis*, is chosen as the type because this is the only one of the five that was known to the older authors as Phalaris.

AIRA L. Sp. Pl. 63. 1753. Linnaeus describes 14 species. The name was first used by Linnaeus in his Flora Lapponica, 1737, where he describes four species, these evidently representing his concept of Aira. From these four the second (*A. caespitosa*, usually known as *Deschampsia caespitosa*) is arbitrarily chosen as the type. To select the first, *A. spicata* (*Trisetum spicatum*), as the type would result in changing the application of the name Aira to the genus now called Trisetum. It causes less confusion to apply the name Aira to the group known as Deschampsia, as is done by many European botanists, than to replace the name Trisetum.

DACTYLIS L. Sp. Pl. 71. 1753. Two species are described: *D. cynosuroides*, now referred to Spartina, and *D. glomerata*, the orchard grass. The second is selected as the type because it was described earlier by the author in his Flora Suecica.

POA L. Sp. Pl. 67. 1753. Linnaeus describes 17 species. From the species described in his Flora Lapponica, *P. pratensis* is selected as the type as this is a familiar, widely distributed, and economic species.

The above examples illustrate the method employed in the selection of types. It has been intended to consider all the factors in each case and to determine if possible what species represented best the author's concept, or, in case two or more species are equally available for consideration, to select the type in such a way as to cause the least confusion in our nomenclature. Aside from Linnaean genera there are comparatively few cases where the evidence does not lead to a definite species as the type. In these few cases an arbitrary selection must be made in such a way as to cause the least confusion in the application of names.

The type of a genus having been fixed it behooves subsequent authors, who would divide genera, to retain the original name for that part which includes the type species.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C

AXILLARY CLEISTOGENES IN SOME AMERICAN GRASSES

AGNES CHASE

A few years ago a previously unknown form of cleistogene was discovered in autumnal specimens of *Triplasis purpurea*.[1] These were solitary, sessile, single florets without glumes, and were borne in the lower sheaths, clasped in the wings of an indurate prophyllum (fig. 1). It was noted that specimens bearing these cleistogenes readily disjointed at the nodes. With this character and a slight swelling above the nodes as clues, other examples were sought from time to time with the result that some twenty more grasses were found to produce similar cleistogenes. They are produced by all the species native in the



FIG. 1. *Triplasis purpurea*. Ordinary spikelet (chasmogene) and cleistogene and grain of each—× 5.

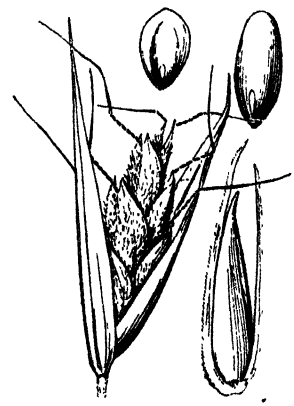


FIG. 2. *Danthonia spicata*. Ordinary spikelet and cleistogene, with subtending prophyllum, and grain of each—× 5.

United States of three genera, Triplasis with three species, Danthonia with twelve, and Cottea with one. They are also found in *Muhlenbergia microsperma* and in *Pappophorum Wrightii*. In all cases the cleistogenes, borne at the lower nodes of flowering culms and not in leafy shoots, are strikingly different from the chasmogenes (that is,

[1] See Bot. Gaz. 45: 135–136. 1908.

the spikelets borne on the terminal panicle) of the same plant. Often, if their source were unknown, they would not be placed in the same tribe. The characters that are common to all are simplified structure and enlarged grain. In Triplasis the prophyllum is enlarged and indurate, and enfolds the entire spikelet; in Danthonia it is thin in texture, is split to the base, and simulates a pair of narrow glumes. (Repeated dissections have been made to decide whether these organs are parts of a prophyllum or a pair of glumes. In the very few immature spikelets found they are evidently prophylla, but immature spikelets are very difficult to find. The keels are ciliolate as are the prophylla, instead of glabrous as are the glumes of the chasmogenes.)

The type of spikelet characteristic of the genus Danthonia is shown in the sketch of *Danthonia spicata*, our commonest species (fig. 2). The two long glumes exceeding the several crowded florets, the short rachilla joints, and the 2-toothed lemma bearing a flattened awn tightly twisted below, are the distinguishing characters of the genus. The cleistogenes are all without glumes. In *Danthonia spicata* there is but one floret, sometimes with a slender rachilla joint bearing a minute rudiment of a second floret. The lemma is not toothed; it is usually merely pointed, but a few have been found with the point slightly lengthened, flattened, and somewhat twisted. (Note the relative size and shape of the grains). In some of the other species, especially the western *Danthonia intermedia*, *D. americana*, and *D. californica*, there are commonly two, three, or four widely separated florets, with slender rachilla joints almost as long as the florets, forming a striking contrast to the crowded florets characteristic of the genus. The lemmas are entire and awnless, or awn-tipped as in *D. spicata*. The genus Danthonia comprises something over 100 known species, Africa being the home of more than half of them. All the material of the genus in the National Herbarium was examined. (The examination, because it necessitates some injury to the specimens, was by no means thorough.) The one Mexican and two West Indian species show no sign of cleistogenes; their wiry junciform habit would not lead one to expect them. Of the South American species, four, *Danthonia chilensis*, *D. cirrata*, *D. montevidensis*, and *D. picta*, were found with cleistogenes. But only one species from the eastern hemisphere, *Danthonia semiannularis* of New Zealand, was found to produce them. From our own species it would appear that these cleistogenes are produced after the maturity of the panicled spikelets. In *Danthonia*

spicata few herbarium specimens reveal these cleistogenes, but if the curly tufts so common in late autumn on sterile knolls and rocky hillsides be examined they are invariably (in my experience) found with cleistogenes in the broken culm or in the internodes remaining in the tuft. I surmise they are a regular rather than an occasional method of reproduction.

The chasmogamous spikelet of *Cottea pappophoroides* is a highly specialized one, the florets being deeply 5- to 7-cleft and awned, and partly hidden in copious white hairs. The cleistogene is usually solitary, without glumes, and consists of a single floret with a length-

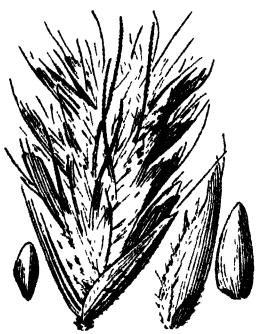


FIG. 3. *Cottea pappophoroides*. Ordinary spikelet and cleistogene and grain of each—× 5.



FIG. 4. *Pappophorum Wrightii*. Ordinary spikelet and cleistogene and grain of each—× 5.

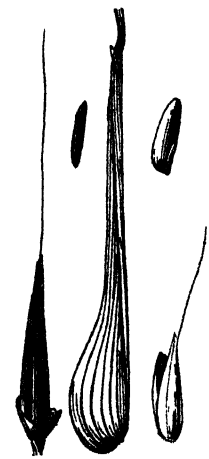


FIG. 5. *Muhlenbergia microsperma*. Ordinary spikelet, cleistogene enclosed in subtending leaf, cleistogene removed, and grain of each—× 5.

ened rachilla joint bearing a minute rudiment of a second floret. The lemma is sparingly silky villous and minutely awned or awnless (fig. 3; note the relative size and shape of the grains). The prophyllum is delicate and split as it is in Danthonia. In a single case a spike of three one-flowered spikelets was found. In these the second glume was developed, and this infolded the floret and rachilla joint.

In *Pappophorum Wrightii* the chasmogamous spikelet bears a

single perfect floret and two or three sterile florets, the lemmas of each being split into nine spreading plumose awns, all the awns together forming a feathery, pappus-like crown. The cleistogene, which is sometimes so rotund as to split the sheath in which it is borne, consists of a floret without glumes, and a rachilla joint with a minute rudiment of a second floret. The lemma is more or less split into a few closely appressed lobes (fig. 4; note the relative size of the grains). The prophyllum is thin in texture and is usually not split. In two cases the cleistogene was infolded in the sheath of a reduced leaf, so judged from its position opposite the prophyllum and from the clearly evident differentiation into sheath and blade. In one case a spike with three one-flowered spikelets was found. In two species of the eastern hemisphere cleistogenes were found, namely *Pappophorum boreale* from Transbaical, Siberia, and *Pappophorum brachystachyum* from the Algerian Sahara.

In *Muhlenbergia microsperma* the chasmogamous spikelet is very small, has minute glumes and a single long-awned floret. The cleistogene is usually awned, without glumes, and much more turgid than is the chasmogene (fig. 5; note the relative size of the grains). It is tightly folded in the swollen, spongy-indurate base of the sheath of a reduced leaf, which sometimes has a minute blade and ligule. In a few cases a tiny raceme was found with three or four spikelets with glumes like those of the chasmogenes. The cleistogenes are produced in abundance at the lower nodes, the subtending sheaths being pushed open by their bulk. (In old plants the numerous little cornucopias may be easily seen at the base.) The subtending prophyllum is membranaceous and remains attached to the nodes. Unlike those of Danthonia and the others, the nodes of *M. microsperma* do not disjoint, but the cleistogenes themselves readily fall at maturity. In Danthonia and the rest, the cleistogenes are permanently enclosed in the sheath together with the internode of the culm. (I surmise that the grains germinate within the sheath and push root and shoot through the internerves, but this has not been proved by experiment.)

In all the species in which these cleistogenes have been studied they are found to be more variable than are the chasmogenes of the same species. Only a limited study can be made in the herbarium, and as yet only Triplasis, Danthonia, and *Muhlenbergia microsperma* have been studied in the field. Notes from observers would be gratefully received. Since with relatively little study so many species have

been found to produce cleistogenes, it seems probable that this is not a rare habit in grasses. Any grass with swollen sheath bases and disjointing culms may repay examination, after the maturity of its terminal panicles. Most of the species so far found are plants of the arid regions or of dry places in the humid regions; all are plants of open ground.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

CYRTANDREAE HAWAIIENSES, SECT. CROTONOCALYCES HILLEBR.

JOSEPH F. ROCK

SECTION TWO: CROTONOCALYCES HILLEBR. FL. HAW. ISL. 325. 1888

Calyx cleft to the middle or less into broad lobes or teeth. Leaves broad, generally rounded or cordate, truncate, peltate or unevensided at the base. Young shoots, leaves, and inflorescence villous with golden or dark brown multicellular hairs.

This section comprised originally eight species and two varieties, *C. cordifolia*, *C. Pickeringii*, *C. honolulensis*, *C. begoniaefolia*, *C. malacophylla*, *C. platyphylla*, *C. Wawrai*, and *C. Kealiae*. Hillebrand's var. *crassifolia* of *C. Pickeringii* which has been raised to specific rank by the writer (*C. crassifolia* (Hillebr.) Rock), and Hillebrand's var. β of *C. platyphylla* (now *C. platyphylla* var. *hiloensis* Rock) were the two recognized varieties. A possible ninth species is C. B. Clark's *Cyrtandra baccifera*. This species is, however, of doubtful validity. It belongs in all probability to some form of *C. platyphylla* A. Gray.

To the species and varieties above enumerated there have been added the following: new species, *Cyrtandra mauiensis*, *C. tintinnabula*, *C. Knudsenii*, and *C. caulescens*; new varieties, *Cyrtandra cordifolia* Gaud. var. *gynoglabra* Rock, *C. mauiensis* Rock var. *truncata* Rock, *C. malacophylla* C. B. Clarke var. *erosa* Rock, *C. platyphylla* A. Gray var. *robusta* Rock, var. *membranacea* Rock, var. *stylopubens* Rock, var. *parviflora* Rock; and one new form, *Cyrtandra platyphylla stylopubens* forma *ovata*. One species, Wawra's *Cyrtandra honolulensis*, has been reduced to a variety as *C. Pickeringii* var. *honolulensis*. It differs from the species only in the pubescent ovary and membranous leaves. One variety, *C. Pickeringii* var. *crassifolia* Hillebr., has been raised to specific rank as *C. crassifolia* (Hillebr.) Rock. Hillebrand's var. β of *Cyrtandra platyphylla* has been given a varietal name, *hiloensis*. *Cyrtandra paritiifolia* Hillebr. was found to be identical with *C. malacophylla* C. B. Clarke, and the latter, an earlier name, was adopted. This brings the Hawaiian species in this section to twelve (possibly thirteen) species, eight varieties, and one form.

For the Hawaiian species the writer has adhered to the sections of Hillebrand rather than those of C. B. Clarke, who places together heterogeneous species and separates related ones. Clarke's section name *Macrosepalae* might be adopted for *C. cordifolia* and *C. Wawrai* and perhaps for *C. tintinnabula* but not for the other species. Much more satisfactory is Hillebrand's grouping based upon depth of division of the calyx.

CYRTANDRA KEALIAE Wawra, Flora 30: 565. 1872

A shrub about 2 m. high; leaves opposite, the upper ones rarely ternate, elliptical-ovate, cuneate at the base or decurrent, acuminate at both ends, on both sides somewhat covered with ferruginous hairs, especially along the midrib and veins underneath, the margin denticulaté, 7–16 cm. long, 4–7 cm. wide, the petioles 2–6 cm. long; flowers solitary in the axils on peduncles 5 mm. long, the pedicels 2 mm. long, the latter bi-bracteate at the base; bracts ovoid-oblong, about 7 mm. long, 3 mm. wide, hirsute with yellowish hairs as are the peduncle and pedicel; calyx urceolate, 14–18 mm. long, 6–7.5 mm. wide, including the 5 mm. long calycine lobes, densely hirsute outside with yellowish-brown hairs 1.5 mm. long, densely villous inside, the silky wool more than 2 mm. in length; corolla twice as long as the calyx, hirsute outside, villous inside; ovary ovoid, glabrous as is the style; fruit 8 mm. long, ellipsoidal.

KAUAI: About Kealia, Wawra no. 2192 in herb. Vienna; Waimea leg. Knudsen no. 203 herb. Hillebr. in herb. Berlin, and in herb. College of Hawaii, no. 13051 ex coll. Hillebr.; Olokele Valley, Abbé A. Faurie, without flower or fruit, March, 1910, nos. 629 and 13052 in herb. College of Hawaii; Olokele Canyon, flowering Oct., 1916, Rock no. 13053 in herb. College of Hawaii; same locality, A. S. Hitchcock, Oct. 18, 1916, nos. 15204 and 15205 in U. S. Nat. Herb.

This species is readily recognized by the densely villous calyx and the short-peduncled single flowers. Wawra's statement is quite correct, that it is difficult to differentiate the reproductive organs owing to the silky, glossy, yellowish-brown hair with which the persistent calyx is completely filled. In the writer's specimens the flowers are not fully developed; the ovary is glabrous but the style is pubescent at the apex only. Wawra states only that the ovary is ovoid, while C. B. Clarke states that the ovary is glabrous with the style. It is doubtful if Faurie's no. 629 belongs here, as the specimen in the College

of Hawaii Herbarium is without flower or fruit. The leaves, however, agree quite well with the typical *C. kealiae*.

Cyrtandra Knudsenii Rock n. sp.

A shrub 2–3 m. high; branches quadrangular towards their apices, stout and hirsute with yellowish hairs; leaves ovate to obovate-oblong, coriaceous, dark green above, dark brown beneath, covered on both sides with dark brown silky appressed hairs, the leaf margin obscurely denticulate and bordered with dense deep golden-brown hairs, acute at both ends, somewhat unsymmetrical at the base, 8–12 cm. long, 3–6 cm. wide, the petioles 2–4 cm. long; peduncles 7–15 mm. long; bracts linear-lanceolate, 6–10 mm. long; pedicels one to three 10–18 mm. long, densely villous-hirsute as are the peduncle, bracts and calyx, the latter unevenly split often to near the base into oblong, acute lobes, 6–20 mm. long, 4 mm. wide, triplinerved, hirsute inside; corolla somewhat protruding, curved, densely hirsute, the lobes oblong; ovary glabrous, ovoid; style nearly 1 cm. long, thickened towards the apex, more or less hirsute.

KAUAI: Halemanu forests, elevation 3,600–4,000 feet, drier forest lands of Kopiwai in company with *Alphitonia excelsa*, *Platydesma cornutum*, *Antidesma platyphyllum*, *Cyanea leptostegia*, *Cyanea hirtella*, etc., flowering Feb. 16, 1909, Rock no. 1688, type in herb. College of Hawaii; Kaholuamano, drier forest, flowering Sept., 1909, Rock no. 5603 in herb. College of Hawaii; same locality, flowering and fruiting Oct. 20, 1916, A. S. Hitchcock no. 15360 in U. S. Nat. Herb.

The specimens from Kaholuamano differ somewhat from those of Halemanu in the shorter and consequently less deeply lobed calyx. The Halemanu specimens have the calyx divided almost to the very base; the lobes are somewhat constricted below the middle but not stipitate as in *C. kauaiensis*, neither are they thin and green, but thick and densely hirsute with brownish hairs, the fruit in *Cyrtandra kauiensis* is hirsute at the apex which would indicate a pubescent or hirsute ovary. The bracts in *C. kauaiensis* are minute and filiform, while in *C. Knudsenii* they are linear-lanceolate. The species is related to *C. kauaiensis* Wawra but must be classed with *C. platyphylla* and *C. Pickeringii*.

In the collection made by A. S. Hitchcock on Kauai the writer found a species of Cyrtandra, collected in the type locality, Kaholuamano, which must be referred to *C. Knudsenii* Rock. In Hitchcock's

specimen no. 15360 the fruit is densely hairy, and the rather large calycine lobes are reflexed.

CYRTANDRA MALACOPHYLLA C. B. Clarke in DC. Monogr. Phan. 5: 227. 1883

Cyrtandra paritiifolia Hillebr. Fl. Haw. Isl. 328. 1888.

Branches terete, softly villous; leaves ovate-cordate, minutely denticulate, shortly acute at the apex, 7 cm. long, 5–6 cm. wide, sparsely covered above with multicellular hairs, yellowish tomentose to densely villous beneath, the villous petioles 4 cm. long, peduncle 2 cm. long, few-flowered; bracts 1 cm. long, elliptical, subacute; pedicels 1 cm. long, calyx 12 mm. long, softly villous, deeply 5-fid to near the base, the lobes lanceolate; corolla glabrous outside; ovary and style glabrous; fruit ovoid, elongate, 14–16 mm. long.

KAUAI: Hillebr., herb. Kew, teste C. B. Clarke.

MAUI: West Maui, gulch of Oloalu, flowering Aug., 1870, Hillebr. in herb. Berlin, part of his type (*C. paritifolia*) in herb. College of Hawaii; East Maui, Hamakua, Haleakala, fruiting, Lydgate, herb. Hillebr. in herb. Berlin.

There is no doubt that *C. malacophylla* is identical with *C. paritiifolia*. The only question arises as to the locality mentioned; the specimen cited by C. B. Clarke as the type of *C. malacophylla* is supposed to be from Kauai (ex coll. Hillebr. in herb. Kew). The description answers perfectly to the specimen (ex herb. Hillebr.) in the College of Hawaii Herbarium, which is part of Hillebrand's type of *C. paritiifolia*. Drake del Castillo also unites these two plants (Ill. Fl. Ins. Mar. Pac.).

C. malacophylla is mainly distinguished by the small leaves, deeply and broadly lobed calyx and glabrous corolla.

Hillebrand's specimen in the Berlin Herbarium was originally labeled *Cyrtandra cordifolia* var. *subglabra*.

CYRTANDRA MALACOPHYLLA **erosa** Rock n. var.

A small brittle shrub 1.5 m. high; leaves as in the species, the margin erose-dentate, green and densely tomentose above, yellow and villous beneath especially on midrib and veins; peduncle much longer than in the species, 5 cm. long; bracts ovate, acute, dentate in the upper part; pedicels 1.5–2 cm.; calyx oblong-campanulate, the lobes broad and short, 3–4 mm. long; corolla long-exserted, curved, hirsute in the upper part; ovary glabrous.

MAUI: On the edge of Honokawai gulch, elevation 4,500 feet, boggy forests, West Maui, flowering Aug., 1910, Rock and Hammond no. 8171, type in herb. College of Hawaii.

This variety differs from the species mainly in the hirsute corolla, short-lobed calyx, longer peduncles, and erose-dentate leaves. This variety and *C. cordifolia gynoglabra* seem to be intermediate between *C. cordifolia* and *C. malacophylla.*

CYRTANDRA WAWRAI C. B. Clarke in DC. Monogr. Phan. 5: 228. 1883

Cyrtandra peltata Wawra in Flora 30: 565. 1872. Not Jack, 1825.
Cyrtandra Wawrae Hillebr. Fl. Haw. Isl. 328. 1888.

A branching shrub 3 m. high or less, the young shoots and inflorescence hirsute with pale ochraceous hairs, the stems quadrangular, glabrate; leaves opposite, broad-ovate, acuminate at the apex, dentate, asymmetrical at the base, 20–28 cm. long, 12–16 cm. wide, densely hirtellose above, tomentulose beneath, peltately affixed 2.5–5 cm. above the base, the petioles 7.5–14 cm. long; peduncles 12–25 mm. long, hirsute, stout, bearing from three to fourteen pedicels 30–40 mm. long; bracts large, foliaceous, ovate, about 25 mm. long; calyx urceolate-campanulate, tomentose inside and outside, 25 mm. long, the lobes broad, ovate-lanceolate, half the length of the tube; corolla slightly exserted the lobes small; ovary and style glabrous; berry included in the calyx, globose, glabrous.

KAUAI: Wasserfall, on Hanalei, Wawra, type no. 2002 in herb. Vienna, part of type in herb. College of Hawaii, also Hanalei (leaf only), Wawra, March, 1870; Waimea, Knudsen, two sheets (flowering specimens) in herb. Berlin; Hanalei waterfall, June 24, 1895, Heller no. 2437; Olokele Valley at the head of intake on rock-walls flowering and fruiting, Sept., 1909, Rock no. 5397 in herb. College of Hawaii; Kaholuamano, elev. 3,800 feet along stream bed, fruiting Oct., 1911, Rock no. 13070, in herb. College of Hawaii; Hanapepe Valley, fruiting Dec., 1909, Abbé Faurie no. 601, specimen in herb. College of Hawaii; Olokele Valley, elev. 1400 feet, Oct. 18, 1916, A. S. Hitchcock no. 15207 in U. S. Nat. Herb.

Cyrtandra Wawrai is certainly a well-marked species and is peculiar to Kauai. Heller states: "Described by both Wawra and Hillebrand as a branching shrub. In no case have I seen it branching." When growing at lower elevation on rock wall as for example in Hanalei and Olokele canyons it is unbranched. But the plants from Kaholuamano are several feet in height and are branching shrubs. The specimens from this latter locality are stouter and larger in every way.

CYRTANDRA CORDIFOLIA Gaud. Bot. Voy. Uranie 446, t. 56. 1826

A shrub 1.5–2.5 m. high, freely branching from the base, the young shoots villous with pale ochraceous hairs; leaves opposite, ovate-suborbicular, shortly acuminate at the apex, cordate at the base, sharply dentate or serrate, 15–20 cm. in diameter, membranaceous to chartaceous, the petioles 5–15 cm. long; peduncle 2.5–6.5 cm. long, subumbellately several-flowered; flowers four to ten on pedicels 15–20 mm. long; bracts foliaceous, broadly lanceolate, 1.5–4 cm. long; calyx membranaceous, whitish, villous inside and outside, cup-shaped, 15 mm. high, evenly divided to the middle or beyond into broadly ovate or triangular lobes, rotately expanded when in fruit or even reflexed; corolla villous, broad tubular, straight, about 15 mm. long, the short lobes rounded, nearly equal; ovary and style villous, the latter very short, articulate below the stigma; berry broad-ovoid, pubescent.

In insulis Sandwicensibus, Gaudichaud, altitude 100–300 hex. leg. 1829. Also visit of 1841.

OAHU: In herb. Berlin, ex coll. Gaudichaud, two sheets, visit 1829 and 1841, ex coll. Meyen, leaves only, ex herb. Soc. Hort. London, Ins. Owhyhee, ad montem-Kaah, Macrae, Junio, 1825,[1] Lindley, visit 1832, Woahoo, Bennet Collection, ex coll. Hillebrand, two sheets, Aug., 1870; in herb. Vienna, Wawra, 3 sheets, det. C. B. Clarke, no. 1743; in herb. College of Hawaii, ex coll. Hillebrand, ex Mus. Bot. Berlin, one sheet, flowering specimen, Aug., 1870, Tantalus, flowering Dec. 2, 1906, Otto H. Swezey, no. 12774, Koolau Mts., Wahiawa range, flowering, Aug., 1908, Rock no. 28, Pauoa Valley, flowering, Oct. 24, 1908, Rock no. 702, Palolo Valley, flowerbuds 1915, Rock no. 1198, Nuuanu Pali, fruiting Oct., 1909, Abbé Faurie no. 602; in U. S. Nat. Herb., Schofield Barracks, East Range, flowering July 11, 1916, A. S. Hitchcock, no. 14037.

One sheet in the Hillebrand collection came from the southern slopes of Mt. Haleakala, 1870. This plant is referable to *Cyrtandra cordifolia gynoglabra* Rock. *Cyrtandra cordifolia* is a very distinct species and can never be mistaken. It is confined to the island of Oahu, although related species occur on Maui. The variety *gynoglabra* differs mainly in the glabrous ovary. In *C. cordifolia* the ovary is densely villous.

[1] This plant seems to have been collected on Hawaii and represents *Cyrtandra platyphylla;* the leaves are rounded but not cordate.

CYRTANDRA CORDIFOLIA **gynoglabra** Rock n. var.

Habit of species; leaves ovate to orbicular in outline, acute at the apex, somewhat oblique and cordate at the base, dark green above, hirsute, dirty brown beneath; peduncles shorter, 3.5 cm. long; calyx as in the species, the lobes very broad-triangular; corolla hirsute; ovary glabrous, as is the articulate style.

MAUI: Eastern part, southern slopes of Mount Haleakala in gulch near Kaupo, 5,000 feet, flowering Nov., 1910, Rock no. 8687 in herb. College of Hawaii.

This plant differs from the species occurring on the island of Oahu at a much lower elevation (1,000-1,500 feet) mainly in the glabrous ovary; the calyx lobes are, as in the species, deltoid to broadly triangular-ovate, and the plant agrees in all other respects with *C. cordifolia*. It differs from *C. begoniaefolia* in the cordate leaves and broad calyx lobes, while the lobes of *C. begoniaefolia* are erect, lanceolate, acute. Hillebrand does not state whether the ovary is glabrous or not.

Cyrtandra begoniaefolia occurs, or rather is supposed to occur, at Ulupalakua not far from where *C. cordifolia gynoglabra* grows, but the writer has been unable to relocate it.

CYRTANDRA **crassifolia** (Hillebr.) Rock (See Pl. XVIII)

Cyrtandra Pickeringii β var. *crassifolia* Hillebr. Fl. Haw. Isl. 327. 1888.

A small stout bush 1 m. high, the stem and branches quadrangular, stout, with corky scaly bark, villous only at the apex, glabrous below; leaves ovate to suborbicular, cordate to obliquely cordate, acute to subacuminate at the apex, densely villous on both sides with fulvous hairs, thick-fleshy, with prominent midrib and nerves, the margin irregularly dentate to serrate, 6–8 cm. long, 4.5–8 cm. broad, the hirsute petioles 3–7 cm. long; inflorescence hirsute throughout; peduncle short, 1 cm. long, the bracts linear-lanceolate, acute, 6–8 mm. long; pedicels 12–20 mm. long; calyx as in *C. Pickeringii*, glabrous inside; corolla exceeding the calyx, about 2 cm. long, curved and hirsute, the lobes rounded, of unequal size; ovary ovoid, glabrous as is the style; stigmatic lobes oblong-spathulate, 2 mm. long; fruit ovoid, acute, glabrous.

OAHU: High ridge of Niu Valley, Hillebrand, no specimen extant; summit ridges above Punaluu Mts., windward side, in exposed situation in company with *Lobelia Gaudichaudii*, *Trematolobelia macrostachys*, etc., flowering and fruiting Dec. 24–29, 1908, Rock no. 492; same locality, flowering Aug., 1911, Rock no. 8825 in herb. College of Hawaii.

No specimen of Hillebrand's *C. Pickeringii* β var. *crassifolia* is in Hillebrand's herbarium, nor in any other herbarium so far as the writer is aware. His type was evidently distributed or was lost. The description however answers the writer's material from the summit ridges of Punaluu, as far as the inflorescence is concerned; the leaves have no resemblance to leaves of *C. Pickeringii*, and Hillebrand gives no description of them under his var. *crassifolia*. The plant is totally distinct from *C. Pickeringii* even in habit. It is of much smaller stature and the thick fleshy leaves occupy a vertical position on the horizontally arranged petioles, giving the plant a peculiar aspect.

Cyrtandra mauiensis Rock n. sp. (See Pl. XIX)

A tall shrub 3 m. or more high, the branches quadrangular, with large leaf scars, densely hirsute with brownish hairs towards the apex; leaves large, obliquely cordate at the base, subcaudately acuminate at the apex, thick-coriaceous, pubescent above, tomentose underneath, but villous along the midrib and veins, the margin denticulate-serrate, 15–22 cm. long, 10–14 cm. wide, on hirsute petioles 5–11 cm. long; inflorescence in the axils of the lower leaves; peduncle 3.5–6 cm. long, hirsute; bracts ovate-oblong, acute, hirsute, caducous, 24 mm. long, 12 mm. wide; pedicels several to ten, up to 2.5 cm. long, with additional linear-oblanceolate bracts at the base of some of the pedicels; calyx oblong, nearly 2 cm. long, divided more than one-third its length, or irregularly, into ovate acute lobes, hirsute inside and outside; corolla exserted one half its length, hirtellous outside, ampliate above the middle and straight; ovary elongate, elliptical-oblong, glabrous; style apparently continuous with the ovary, glabrous; fruit glabrous, elliptical, enclosed in the calyx.

MAUI: Honomanu gulch, along lower ditch trail (Kailua), dense wet forest, northern slopes of Mt. Haleakala, elev. 2,400 feet, flowering May, 1911, Rock no. 13028, type in herb. College of Hawaii.

This species is related to *C. malacophylla* C. B. Clarke, but differs from it in the large, ovate-oblong, subcaudately acuminate leaves, large inflorescence, oblong calyx, straight, ampliate, and hirsute corolla.

CYRTANDRA MAUIENSIS **truncata** Rock n. var.

Leaves smaller, ovate-acuminate, but contracted at the base and cuneate-truncate to rounded or subcordate, the petioles as long as in the species but densely strigosely hispid with golden-brown hairs; inflorescence many-flowered; bracts smaller; calyx larger, the two lower lobes oblong-acute, the three upper very short, 3–4 mm. long, the whole calyx irregularly split, villous on both sides; fruit glabrous, ovoid-oblong, the young fruits crowned by the long recurved articulate style, later becoming caducous.

MAUI: East Maui, northern slopes of Mt. Haleakala, along the Kula pipe line trail near Honomanu gorge in dense wet forest, elev. 4,000 feet or more, fruiting Sept., 1910, Rock no. 8548, type in herb. College of Hawaii.

A. S. Hitchcock collected a specimen on Maui, east of Olinda, elevation 4,000 feet, fruiting Oct. 1, 1916, no. 14911, in U. S. Nat. Herb. It differs from the writer's specimens in the much larger leaves (23 cm. long, 15.5 cm. wide), which are emarginate at the base or very shallow-cordate, the petioles 17 cm. long. The inflorescence is the same, but the tomentum or rather pubescence of the whole plant is darker and of a less glossy brown.

Another specimen collected by A. S. Hitchcock on West Maui, Mt. Puukukui, on Sept. 24, 1916, no. 15593 in U. S. Nat. Herb., belongs here. It differs somewhat from the East Maui specimen in the smaller leaves and more evenly lobed calyx, the flowers much exserted. It is close to *C. malacophylla* C. B. Clarke, but the latter has small leaves and a small inflorescence and shorter flowers.

This variety occurs nearly two thousand feet higher than the species. It is easily distinguished by the leaves which are ovate and not ovate-oblong; the base is mainly cuneate to truncate instead of deeply and unevenly cordate. It is in all probability related to *Cyrtandra malacophylla* C. B. Clarke, but differs from it in the larger leaves and large inflorescence, the long peduncle and larger calyx with irregular lobes.

The locality where the species and variety occur were inaccessible until some seven years ago, when these dense forests and gorges were explored for the first time by the writer. A trail had been blazed at that time for surveying purposes.

Cyrtandra tintinnabula Rock n. sp. (See Pl. XX)

A shrub 2 m. or more high, soft-wooded, resembling *C. cordifolia*; leaves opposite, broadly ovate to suborbicular, irregularly dentate and ciliate at the margin, acute or cuspidate at the apex, decurrent to truncate and more or less asymmetrical at the base, never cordate, the first lower pair of veins almost at right angles to the petiole, dark green above, pale beneath, pilose above with five- to seven-celled, articulate transparent hairs, the cells short and broad, pubescent below especially along the midrib and veins with equally transparent 3–5-celled hairs, the joints narrower and much longer, the blades 10–14 cm. long, 7–12 cm. wide, the petioles 3–10 cm. long; inflorescence cymose, fulvous hairy throughout, the peduncle 10–12 mm. long, with two

large broad clasping bracts of irregular shape; flowers usually six: pedicels of unequal length, varying from 5–11 mm.; calyx broad, campanulate, the bell-shaped tube 6 mm. long, the lobes broadly triangular, 3–4 mm. each way, reflexed from the truncate margin of the tube, hirsute inside especially near the margin and lobes, subglabrous towards the base; corolla straight, cylindrical, 12 mm. long, 5 mm. wide, the tube exserted 8 mm., the lobes short, rounded, subequal, glabrous inside, yellowish-hirsute outside, only the lower portion of tube subglabrous to glabrous; ovary surrounded at the base by a glabrous annular disc; ovary glabrous, only part of style and apex of ovary slightly pubescent with scattered multicellular hairs; fruit unknown.

HAWAII: In the forests of Paauhau no. 2, near waterfalls between the crevices of huge boulders, northern slope of Mauna Kea, elevation 3,000 feet, flowering July 3, 1909, Rock no. 3290, type in herb. College of Hawaii.

This interesting species belongs to the group with *C. cordifolia* rather than *C. platyphylla* on account of the triangular, reflexed calycine lobes. It differs from the latter in the subglabrous ovary, smaller calyx and straight cylindrical corolla, the short peduncle and oblique (not cordate) leaves. The name *tintinnabula* refers to the bell-shaped calyx.

CYRTANDRA BEGONIAEFOLIA Hillebr. Fl. Haw. Isl. 328. 1888

Size and habit of *C. cordifolia*; young branches and inflorescence villous with a bright shining pale-fulvous tomentum; leaves opposite, broadly ovate, 17.5–20 cm. long, 8.75–10 cm. wide, inequilateral, oblique, cuspidate, sharply dentate with broad patent teeth, rounded at the base, with one side much more deeply attached than the other to a petiole about 7.5 cm. long, thick chartaceous, hirsute above, tomentose beneath, with ribs and veins villous; peduncle fleshy, 24 mm. long, bearing three or more flowers on pedicels 20 mm. long; bracts foliaceous, ovate-lanceolate, 24 mm. long; calyx villous, thin funnel-shaped, 20 mm. long, divided to the middle or less into erect lanceolate acute lobes; corolla as long as the calyx, villous or pubescent, straight.

MAUI: East Maui, southern slopes of Mt. Haleakala, Ulupalakua, flowering Sept., 1870, Hillebrand in herb. Berlin, part of type in herb. College of Hawaii.

There is only one sheet of this species in the Berlin Herbarium (ex coll. Hillebr.). It was originally labeled by Hillebrand "*Cyrtandra triflora* Gaud. var. *β arguta folia unequilateralibus*." The place known as Ulupalakua on East Maui must have been more or less covered with forest in Dr. Hillebrand's days. Today there is nothing but

meadow land and planted Eucalypti. Many plants which were peculiar to that region, as for example *Cyanea comata*, *Cyanea arborea* and others, have vanished forever and among them is also *Cyrtandra begoniaefolia*. This species could only have thrived in dense shady forests,[2] which today are no more and their place is taken by a cattle ranch, covered with obnoxious weeds. The writer is acquainted with *C. begoniaefolia* only from the single sheet in the Hillebrand collection. It differs mainly from *C. Pickeringii* to which it is related, in the oblique, not cordate leaves.

CYRTANDRA PLATYPHYLLA A. Gray, Proc. Amer. Acad. 5: 350. 1862

Plant about 3.5 m. high; leaves subrotund to cordate at the base, shortly acuminate, denticulate, 12–20 cm. long, slightly less wide, shortly and densely yellowish-pilose above, pubescent to subvillose beneath, the petioles 3–6 cm. long; peduncles 3 cm. long, many flowered, the bracts never clasping; pedicels 1 cm. long; calyx at flowering about 1 cm. long, irregularly 5-fid, the lobes oblong-lanceolate or broadly lanceolate, shorter than the corolla; ovary and style glabrous; fruit narrow-oblong, sessile.

HAWAII: In forests, U. S. Exploring Exped.; Hilo forests, Hillebrand, one sheet ex coll. Hillebrand in herb. Berlin; forests near the Volcano of Kilauea, elev. 3,800 feet, Kalanilehua, flowering Aug., 1917, Rock no. 12990 (*typica*); Naalehu forest, Kau, elev. 3,500 feet, flowering Jan., 1912, Rock no. 10030 in herb. College of Hawaii; Kohala Mts., Alakahi—Kawainui ditch trail, flowering July 13, 1909, Rock no. 4474 in herb. College of Hawaii.

Cyrtandra platyphylla with its varieties and forms is certainly the predominating species on the island of Hawaii. In fact it is the most variable species of Cyrtandreae in the Hawaiian Islands. This tends to show that it is still in the process of evolution, as is the case with certain species of Lobelioideae on the same island. It may be stated that Hawaii has fewer species of *Cyrtandra* than any other island of this archipelago. There are a few arborescent forms and one or two herbaceous ones as *C. paludosa*, but nothing like the number of species, really distinct species, that occur on Oahu or Molokai.

Kauai possesses about the most settled species, but they are few in number compared to those occurring on Oahu and Molokai. What is lacking in species on Hawaii is there made up in varieties and forms of this variable species "*Cyrtandra platyphylla*," which is almost the

[2] Rock, Indigenous Trees Haw. Isl. page 21, also plate 145.

despair of the systematist who wishes to bring some order into this group.

Cyrtandra platyphylla is evidently the outcome of *Cyrtandra cordifolia, C. tintinnabula, C. malacophylla*, etc. The latter occurs on West Maui (the older portion of Maui) and *C. tintinnabula* on Hawaii proper in an older section of the island, while *C. cordifolia* occurs on Oahu and a variety of the same, var. *gynoglabra*, on East Maui, that part of Maui nearest to Hawaii. The glabrous ovary of var. *gynoglabra* brings it close to *C. platyphylla*, while otherwise it has all the characters of *C. cordifolia* from which it cannot be separated. We find several links here between these species; for example, variety *stylopubens* of *C. platyphylla* has a glabrous ovary and a hirtulous style, while *gynoglabra* of *C. cordifolia* has a glabrous ovary and style which brings it closer to *C. platyphylla*, while the former would appear to be also a link between the two species mentioned, namely, *C. platyphylla* and *C. cordifolia*. *C. malacophylla* seems to be nothing more than an intermediate occurring on Maui, an intermediate island, while *C. Pickeringii* seems to be some sort of an offspring of *C. cordifolia*, and *C. Garnottiana*. The species with the most villous ovary is *Cyrtandra kealiae* which occurs on Kauai, the oldest island of the group. *C. cordifolia* comes next on Oahu, and finally we reach *C. platyphylla* with a perfectly glabrous ovary and style.

Cyrtandra platyphylla, as in the case of other plants belonging to rather large families or representatives of such, occurring on Hawaii, are prone to variation. There are a great many varieties and forms which must be classified in some way. Asa Gray states, "*Leaves subrotund-cordate at the base*," while Hillebrand states, "*suborbicular, ovate-oblong, rounded or sometimes subcordate but oftener contracting at the base*." Hillebrand in his key bases the distinction between *C. platyphylla* and his *C. paritiifolia* (*C. malacophylla*) on the cordate leaves in the latter, and rounded or decurrent leaves in the former. The typical form collected by Pickering in the forests of Hawaii (and not Oahu), which the writer examined in the Gray Herbarium, has decidedly cordate and not decurrent leaves. The writer has at his disposal a large amount of material apparently referable to *C. platyphylla* according to Hillebrand's description of the species. It seems advisable, however, to separate the decurrent leaved forms from the typical one with cordate leaves, which is not uncommon around the Volcano of Kilauea where Pickering in all probability collected the material which served as the type for *C. platyphylla*.

The species is very common all over Hawaii, if we regard it as a variable species. The typical *C. platyphylla* was collected by the writer in the forest near Kilauea Volcano on Hawaii. As the description by Asa Gray is rather brief especially in regard to inflorescence, the following may serve as the diagnosis for

CYRTANDRA PLATYPHYLLA **typica** Rock (See Pl. XXI)

Leaves large, suborbicular, rounded or cordate at the base, shortly acuminate at the apex, 12–20 cm. long and wide, the petioles 3–6 cm. long, pubescent to hirsute on both sides with yellowish-brown multicellular, transparent hairs, especially so on midrib and veins as well as petioles, the latter densely hirsute, the hairs darkening to deep brown near the equally brown hirsute stem; inflorescence hirsute, the peduncle 4 cm. long, bearing at the apex two obovate acute sessile bracts, the latter 20 mm. long, 8 mm. wide, and several-nerved; two distinct cymes at the end of this common peduncle, each bearing three to four long-pedicellate flowers, making the inflorescence subumbellate; between these two cymes two single, long-pedicellate flowers, the lateral cymes possessing a secondary peduncle 1 cm. long, and pedicels varying in length from 12–20 mm.; the central pedicels without secondary peduncle being 25 mm. long; calyx unequally divided into five lobes, hirsute on both sides; corolla tube about 15 mm. long, cylindrical, straight, hirsute outside, with the exception of the lower portion, exserted, the lobes rounded, spreading and subequal, glabrous inside as is the tube; ovary oblong; style, including the very small, bilobed stigma, 5 mm. long; ovary and style absolutely glabrous.

HAWAII: In forests, U. S. Explor. Exped., in Gray Herbarium; forests near Kilauea Volcano, flowering Aug., 1917, Rock no. 12990 in herb. College of Hawaii.

The plants coming nearest the typical form described above are:

CYRTANDRA PLATYPHYLLA **stylopubens** Rock n. var.

Leaves suborbicular, coarsely serrate, 12 cm. long, 10–12 cm. wide, rounded (not cordate) at the base, shortly acuminate at the apex, sparingly hirtulose on the upper surface, the transparent cellular hairs caducous on the older leaves, only very sparingly hirtulose underneath, thin-papery; petioles 3.5–5 cm. long, hirsute; inflorescence a few (three-) flowered cyme; common peduncle 10–12 mm. long, bearing at the apex a pair of subfoliaceous, ovate-acute bracts, 1 cm. wide and 2 cm. long; pedicels about 15 mm. long when with flower, 20 mm. when with fruit, hirsute throughout with dense pubescence, the hairs 1.5 mm. long, horizontally spreading, the pedicels dilating near the calyx; calyx thin, submembranaceous, green, but with numerous scattered brownish hairs; the lobes lanceolate-oblong, irregular in length and

width, each lobe with a strong median nerve and often two lateral ones, 10 mm. long, 5 mm. wide, acute; corolla scarcely exserted, curved, pubescent outside only to half its length, but distinctly and prominently nerved, entirely glabrous inside; ovary ovate, glabrous; style distinctly articulated near the ovary, pale brown and swollen at the base (obclavate), and hirtulose in the upper three fourths.

HAWAII: Forest of Paauhau no. 3, northern slopes of Mauna Kea, elev. 4,000 feet, flowering July 5, 1909, Rock no. 4064, and July 6, 1909, Rock no. 4493, type in herb. College of Hawaii.

The variety *stylopubens* differs from the species in many ways, but especially in the obclavate pubescent style, almost hidden corolla, in the short peduncle, and large calyx lobes. The leaves are not cordate but rounded at the base. The ovary, instead of being oblong and continuous with the style, is ovate and constricted at the base of the articulate style. The plant is almost worthy of specific rank.

CYRTANDRA PLATYPHYLLA STYLOPUBENS forma **ovata** Rock n.f.

Leaves ovate-oblong, acuminate, subglabrous above, with a scattered pubescence underneath, especially along midrib and veins, the margin serrate, often coarsely serrate to near the contracted base; inflorescence as in the preceding; calyx thin, membranaceous, green, pubescent outside, glabrous inside, the lobes strongly one-nerved; corolla slightly protruding, the lobes larger and rounded; fruit oblong, the calyx persistent and enclosing the fruit, but the lobes deeply cut to near the base when with fruit.

HAWAII: Paauhau Forest no. 3, flowering and fruiting July 6, 1909, Rock no. 4495 in herb. College of Hawaii.

This form of variety *stylopubens* differs from the latter in the oblong leaves, and in the larger calycine lobes which are glabrous inside; the leaves are coarsely serrate to near the base.

CYRTANDRA PLATYPHYLLA **parviflora** Rock n. var. (See Pl. XXII)

A shrub 3–4 m. high; leaves large, 25–28 cm. long, 10–14 cm. wide, thin, chartaceous, bluntly serrate to denticulate, ovate-oblong, acuminate at the apex, acute and contracting at the base, dark green above, very sparingly hirtulose, pale yellowish-brown beneath, finely and densely velvety-pubescent, the pubescence short; petioles 7–10 cm. long; inflorescence a three- to five-flowered cyme, hirsute with brownish-yellow multicellular hairs; peduncle about 2.5 cm. long, bibracteate at the apex, the bracts linear-oblong, distinctly three-nerved, 2–2.5 cm. long, 4–5 mm. wide, the pedicels 12–25 mm. long; calyx, including the lobes, 1 cm. long, the lobes lanceolate, acuminate, of unequal length, hirsute outside, glabrous inside; corolla small, the tube cylindrical,

curved, 8–10 mm. long, 2–3 mm. wide, the lobes small, rounded, hirsute outside, glabrous inside; ovary glabrous, as is the style; stigma pubescent beneath the lobes; fruit ovoid to obovoid, scarcely protruding from the calyx.

HAWAII: Forests of the Kohala Mountains, Kohala proper, flowering July, 1910, Rock no. 10339, type in herb. College of Hawaii.

The plant differs from the species and the var. *stylopubens* in the large leaves and mainly in the very small flowers which are nearly hidden in the calyx. The calyx as well as lobes are glabrous inside. The bracts, instead of being ovate or broadly obovate, are linear to lanceolate-oblong.

CYRTANDRA PLATYPHYLLA **membranacea** Rock n. var.

Leaves ovate to ovate-oblong, acuminate at the apex, rounded to contracted at the base, the petioles 4–7 cm. long, thin membranaceous to subchartaceous, 12–14 cm. long, 6–8 cm. wide, hirtulose on both sides, dark above, pale beneath; inflorescence cymose, consisting of a peduncle 2–3.5 cm. long, and pedicels varying from 1–2 cm. long, the latter especially pubescent with yellowish hairs towards the base of the calyx; calyx thin-membranaceous, green, hirsute with scattered hairs; corolla pubescent outside; ovary and style glabrous.

HAWAII: Woods above Waimea in swampy forest, elev. 3,000–3,500 feet, flowering and fruiting July 6–10, 1909, Rock no. 4497 and 4078 respectively, the latter specimen from Holokaiea gulch back of Waimea. Type 4078 in herb. College of Hawaii Herbarium; Waimea, flowering Aug. 26, 1916, A. S. Hitchcock no. 14368 in U. S. Nat. Herb.

Differs from the species in the membranaceous leaves which are contracted at the base. Only a single fruit seems to develop from an inflorescence; the bracts are caducous and the junction between the peduncle and pedicel is often not discernible, which gives it the appearance of having single-flowered continuous pedicels (no. 4497). Here also belongs no. 13066, collected on the southern slopes of Mauna Loa, in the forests of Malehu, Kau; the plant agreeing exactly with those from Waimea of the same island save in the pedicels which are shorter.

CYRTANDRA PLATYPHYLLA **hiloensis** Rock

Cyrtandra platyphylla A. Gray β var. Hillebr. Fl. Haw. Isl. 329. 1888.

"Peduncle and pedicels only 8–12 mm. long; calyx and corolla much shorter, the former less deeply lobed, the latter curved and exserted; fruit short-ovoid, enclosed in the calycine tube; leaves thick, with strong and straight nerves."

HAWAII: Woods of Hilo, leg. Lydgate, in herb. Berlin, part of type in herb. College of Hawaii, no. 13065. This variety was not collected by the writer.

CYRTANDRA PLATYPHYLLA **robusta** Rock n. var.

Plant stout, robust, the stems strongly quadrangular and of almost even thickness to the apex, densely villous-hirsute, with dark reddish-brown hairs; leaves ovate-oblong, acute or acuminate, with denticulate margins, covered with multicellular hairs on the upper surface, densely villous-tomentose beneath, as are the petioles; peduncle about 3 cm. long; pedicels 1.5–2.5 cm. long, three-flowered, densely villous as are the obovate, acute bracts and calyx, the latter subequally divided to beyond the middle into oblong acuminate lobes, which are hirsute on both sides; corolla slightly protruding, the small rounded lobes glabrous on both sides, with the exception of the margin which is slightly ciliate, the tube hirsute nearly to the base, but more so in the upper part; ovary glabrous; style hirtulose with scattered multicellular hairs.

HAWAII: Kawainui ditch trail, Kohala, dense rain-forest, elev. 3,500 feet, flowering June, 1910, Rock no. 8317, type in herb. College of Hawaii.

This variety, which has the hirtulose style in common with var. *stylopubens* of the same species, differs from that variety in the very stout quadrangular stems, dense villosity, longer peduncles, more robust inflorescence, and in the thick calyx lobes which are densely hirsute on both sides.

CYRTANDRA BACCIFERA C. B. Clarke in DC. Monogr. Phan. 5: 228. 1883

The writer is not acquainted with this plant, nor has he seen the type. When the type will have been examined it will undoubtedly prove to be only a variety of *Cyrtandra platyphylla*. Clarke's description may answer any of the forms of that species, as for example "*folia opposita, pedunculi saepe oppositi*," etc.; there is nothing definite in the description whereby it could readily be distinguished from *Cyrtandra platyphylla*. In conclusion he states: "*species C. Pickeringii forsan affinior, ab hac differt (inter alia) ovario baccaque glabris*"; this however is one of the main differences between *C. Pickeringii* and *C. platyphylla*. It is true that the former has broadly triangular calyx-lobes, but those of the latter species are exceedingly variable, and calycine lobes as described by C. B. Clarke in *C. baccifera*, "late lanceolati," occur certainly in *C. platyphylla*.

Cyrtandra caulescens Rock n. sp. (See Pl. XXIII)

Stem somewhat prostrate, terete in the lower portion, subquadrangular in the upper, hirsute with dark brownish hairs; leaves membranaceous to chartaceous, ovate, acute at both ends, light green above, paler underneath, the margins irregularly serrate to dentate, hirsute on both sides, about 15 cm. long, 8 cm. wide, the petioles 4.5 cm. long; inflorescence a much-branching hirsute cyme, not proceeding from the leaf-axils, but from the prostrate stems near the roots, or somewhat above the ground, in dense shade and almost hidden; the two common peduncles opposite each other, around old leaf scars, about 12 mm. long; flowers numerous, on short pedicels; bracts small, lanceolate, 6 mm. long, 1 mm. wide; calyx tube 5 mm. long, campanulate, hirsute with brownish hairs, the truncate broad lobes 3–5 mm. long, 2.5–3 mm. wide, hirsute inside and outside; corolla 8 mm. long, urceolate, constricted at the middle, hirsute in the upper two-thirds only, the lobes small, rounded, hirsute; ovary glabrous.

MAUI: East Maui, dense forest of Hamakua, elev. 4,000 feet, on the banks of a stream near Honomanu gorge, flowering Sept., 1910, Rock (type) no. 8556 in herb. College of Hawaii.

This species is very remarkable for its densely glomerate inflorescence which is borne along the woody portion of the plant near the ground and often on protruding roots. In that respect it comes close to another new species found in deep ravines on Oahu along streams, belonging to a different section. The Oahu species has the flowers on long branching racemes, but they are also borne on protruding roots and along the lower portion of the stem.

CYRTANDRA PICKERINGII A. Gray, Proc. Amer. Acad. 5: 350. 1862

A shrub, the branches densely ferruginous to reddish villous or subhirsute; leaves ovate, oblong-lanceolate, acuminate at both ends or the base cuneate or almost round, serrate, up to 16 cm. long, 5–9 cm. wide, villous on both sides but sparingly so on the upper surface, pale yellowish-brown on the lower, the petioles 2–6 cm. long; peduncles 1–2 cm. long, three- to seven-flowered; bracts 12 mm. long, free, ovate-lanceolate; cyme somewhat loose, simple to dichotomous, pedicels 1–3 cm. long; calyx 7 mm. long, divided to the middle into four to five lobes, the lobes oblong to subovate, persistent; corolla 12–15 mm. long, straight, villous outside; ovary glabrous or slightly pubescent, the style glabrous, clavate; fruit 12 mm. long, ellipsoidal, densely but shortly villous.

OAHU: Mountains of Oahu, Hillebrand no. 322 in herb. Kew, in herb. Berlin and in herb. College of Hawaii; Koolau Mts., Punaluu ridges, flowering Dec. 3–14, 1908, Rock no. 763, same locality Dec.

24–29, 1908, flowering, Rock no. 388, in herb. College of Hawaii; Kalihi Valley, flowering, Aug. 2, 1916, A. S. Hitchcock no. 14105, in U. S. Nat. Herb.

KAUAI: Around Pohakupili, Wawra no. 2191 (*foliis lanceolate-ellipticis*), in herb. Vienna (teste C. B. Clarke).

The form mentioned by C. B. Clarke under *C. Pickeringii* is referred to that species on C. B. Clarke's authority. The writer has not seen this plant nor has he collected any plants on Kauai which could be referred to *C. Pickeringii*.

In the writer's material of *Cyrtandra Pickeringii* the ovary as well as style is glabrous. In Hillebrand's plant the ovary and style are slightly pubescent; the leaves of his plants are large, measuring 16 cm. in length, and are rounded at the base. The leaves in the writer's plants from the Punaluu mountains are acute or acuminate at both ends as called for in the original description; the inflorescence is a lax cyme with peduncles 3.5 cm. long and pedicels of variable length up to 2.5 cm.

Hillebrand classes *C. honolulensis* Wawra with *Cyrtandra Pickeringii* A. Gray. It is true that the former is very close to the latter; Wawra himself says that he is at a loss exactly where to place this plant as it forms an intermediate between *C. Pickeringii* and *C. Lessoniana*. C. B. Clarke upholds Wawra's species and places it even in another section. The plant is not specifically distinct but may well be considered a variety of *C. Pickeringii* A. Gray. (See *C. Pickeringii honolulensis* (Wawra) Rock.)

CYRTANDRA PICKERINGII **waiheae** Rock n. var.

Cyrtandra Pickeringii forma *ovato-elliptica* Wawra, Flora 3: 564. 1872

Leaves ovate-elliptical, chartaceous, acuminate at the apex, rounded at the base, slightly asymmetrical, coarsely and irregularly serrate, pilose on both sides, 10 cm. long, 6 cm. wide, the petioles 1 cm. long; cyme with one or three pedicels; peduncle 3 cm. long, hirsute; pedicels of the same length, bracts small, ovate, 10–12 mm. long; calyx campanulate, thin, membranaceous, the lobes short, acute; fruit ovate, glabrous.

MAUI: "Um Waihee," Wawra no. 1819 in herb. Vienna, part of type in the herb. College of Hawaii.

This variety is here referred to *C. Pickeringii* with some hesitation. The material is rather fragmentary, and in absence of flowers cannot be determined more definitely. It is distinct from *C. Pickeringii*,

and differs from it in the thinner leaves, which are coarsely serrate and in the membranaceous calyx. The ovary is glabrous as in the species.

CYRTANDRA PICKERINGII **honolulensis** (Wawra) Rock

Cyrtandra honolulensis Wawra, Flora 30: 567. 1872.

Cyrtandra Pickeringii A. Gray; Hillebr. in part, Fl. Haw. Isl. 327. 1888.

A much branching shrub; leaves subcoriaceous, ovate, shortly acuminate at the apex, rounded at the base, serrulate, sparsely covered with whitish hairlets at the upper surface, whitish-yellow-silky beneath, 10 cm. long, 5–6 cm. broad, the petioles 4 cm. long; peduncle slender, one to several-flowered, pedicels 0–15 mm. long; bracts 0-1 cm. long, obovate-oblong, obtuse; calyx crateriform, 5-fid, the lobes triangular, acute; corolla 2 cm. long, straight, broadly cylindrical; ovary and style hirsute.

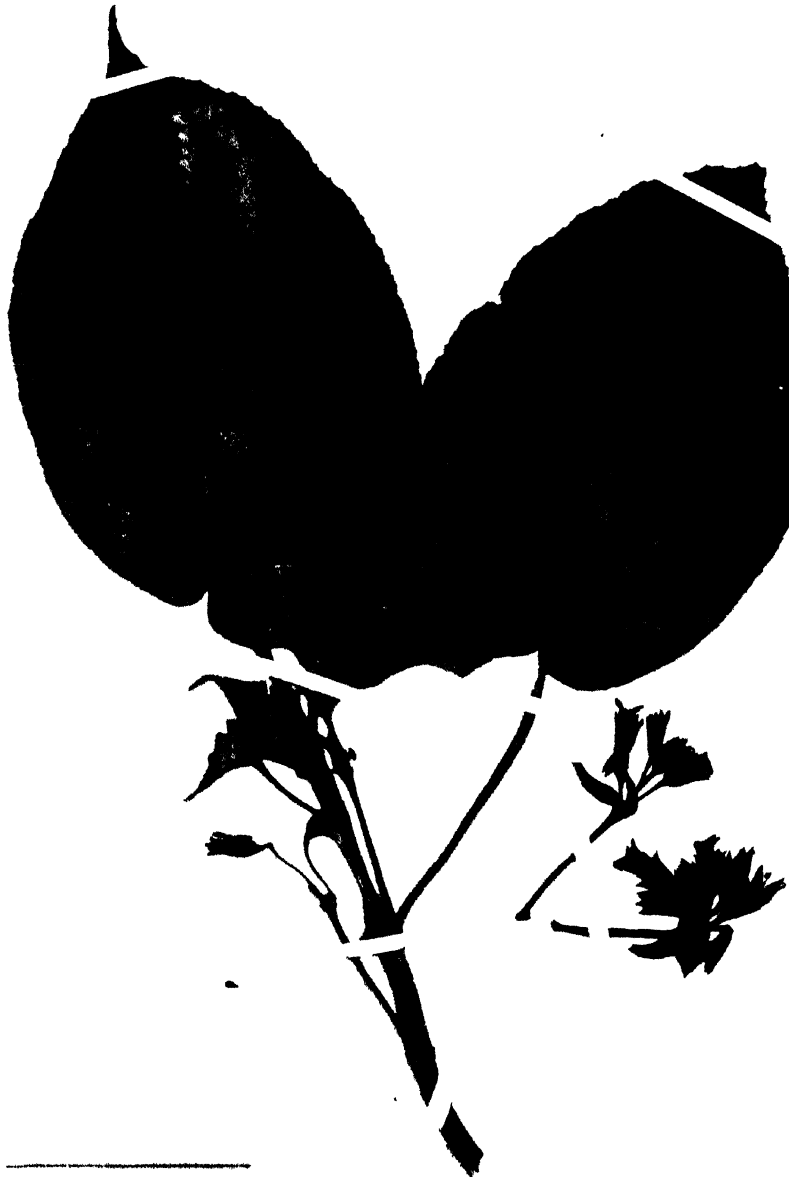
OAHU: Near Honolulu, Wawra no. 1720 in herb. Vienna, part of type in herb. College of Hawaii; Hillebrand no. 328 in part, herb. Kew; Koolau Mts., Punaluu, flowering, Aug., 1908, Rock no. 10 in herb. College of Hawaii.

This variety differs from *C. Pickeringii* A. Gray mainly in the hirsute ovary and style; the cymes are not lax, but usually single to three-flowered. The leaves are thinner, almost membranaceous (when dry). It is not uncommon in the mountains behind Honolulu.

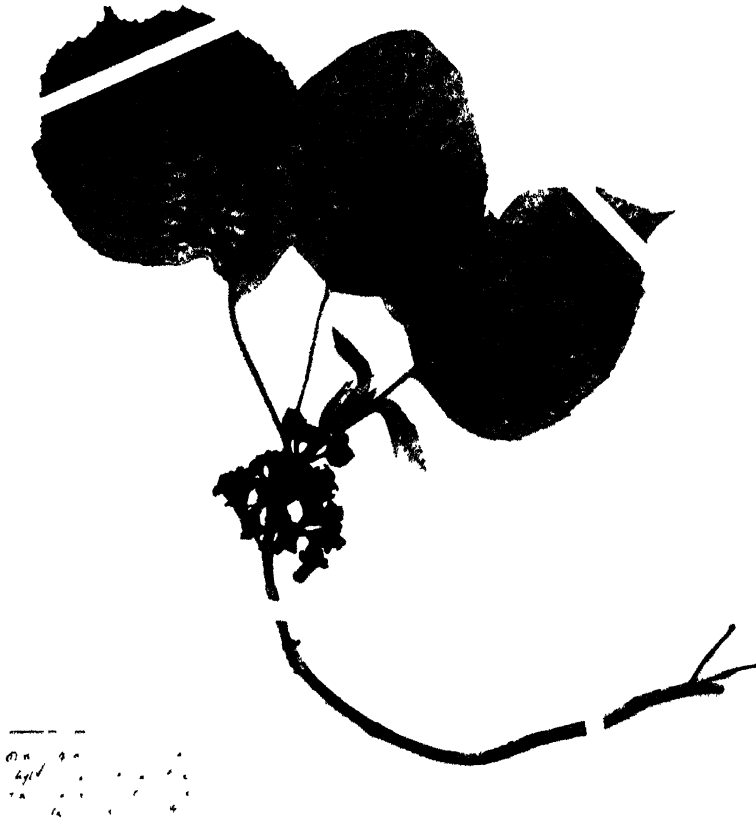
COLLEGE OF HAWAII, HONOLULU



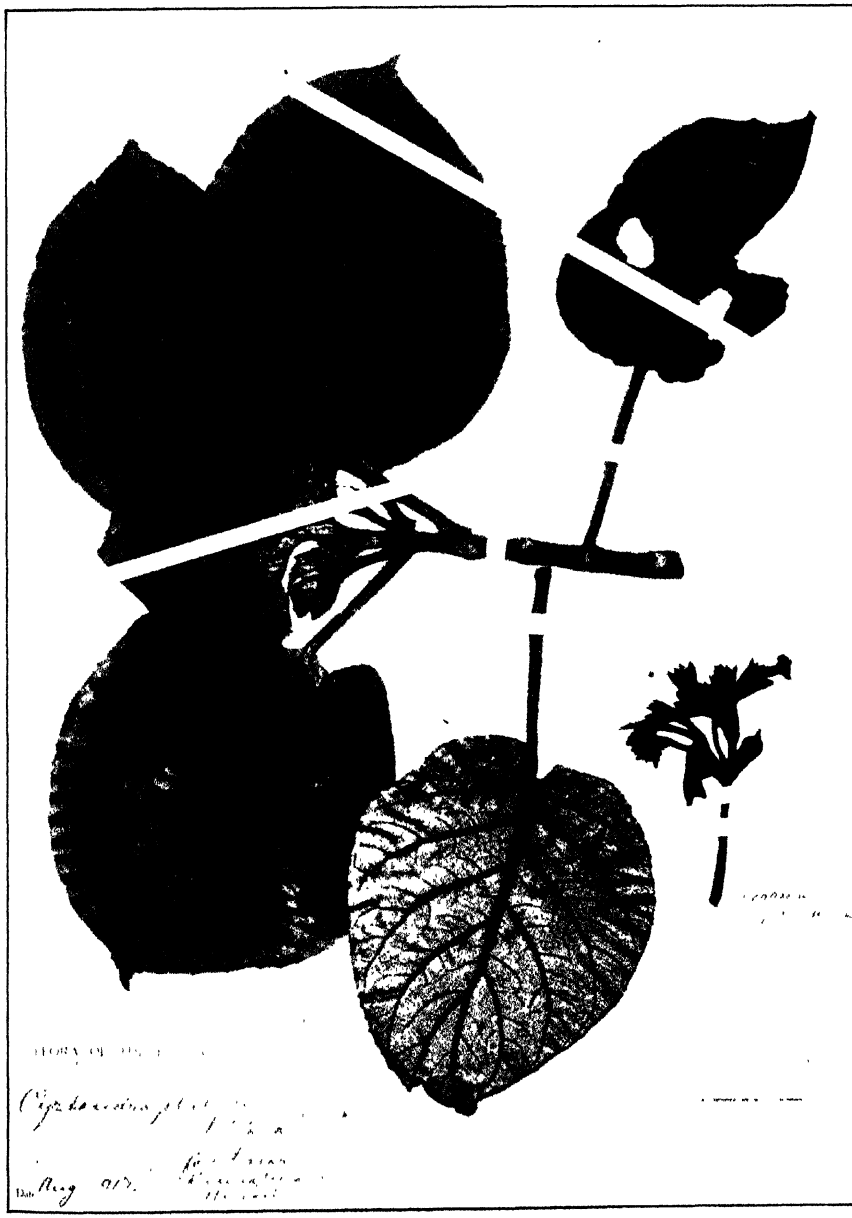
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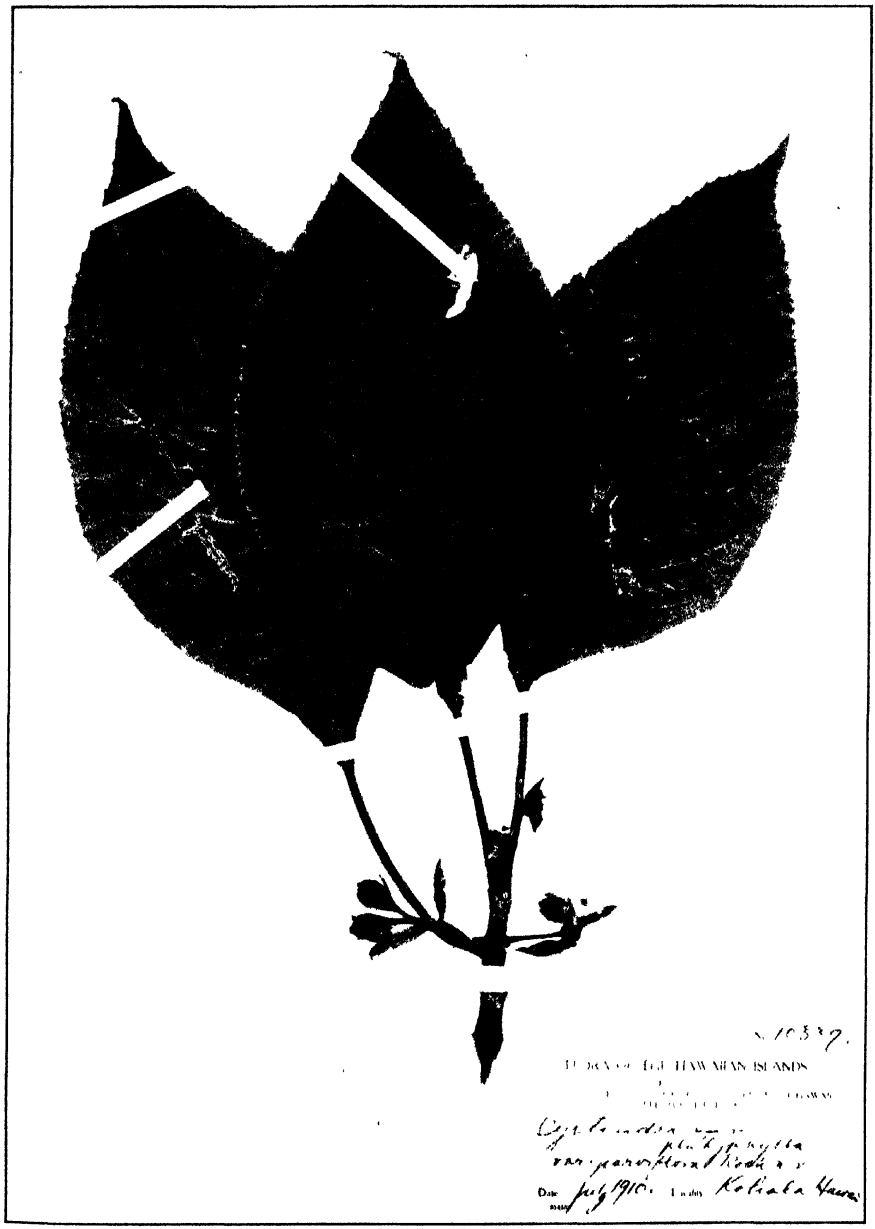
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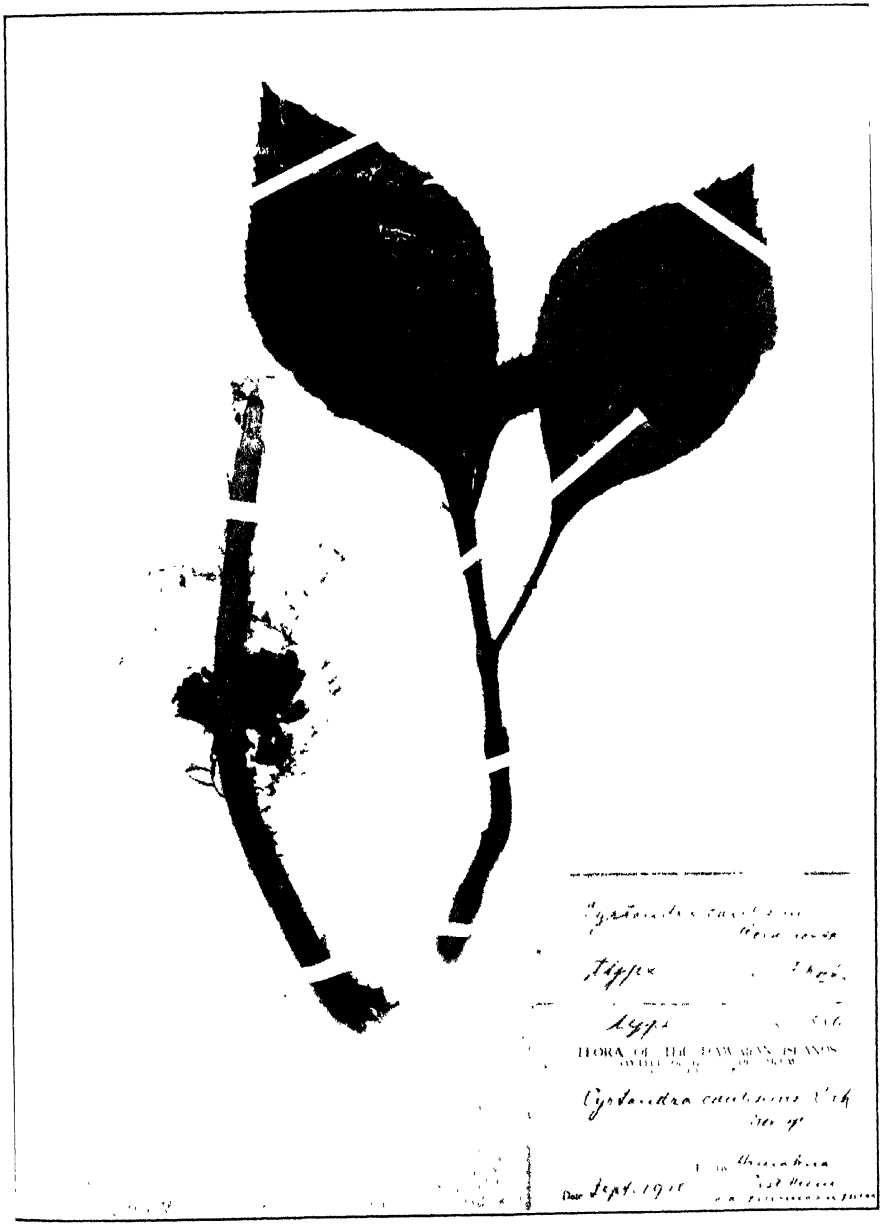
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ROCK: CYRTANDRA PLATYPHYLLA TYPICA ROCK



ROCK: CYRTANDRA PLATYPHYLLA PARVIFLORA ROCK



ROCK: CYRTANDRA CAULESCENS ROCK

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BREEDING FOR DISEASE RESISTANCE IN PLANTS[1]

W. A. ORTON

Our common asparagus (*Asparagus officinalis*) was cultivated in America from the time it was introduced from Europe until 1896 without any losses from disease worthy of record. The varieties in general use were then of American origin and had been developed for qualities other than disease resistance.

Asparagus rust, a disease due to the parasitic fungus *Puccinia asparagi*, was discovered in New Jersey in 1896, and within six years had spread to the Pacific coast. This rust proved very destructive; asparagus fields were killed within a few years. Attempts to control it by spraying or other preventive and sanitary measures were unprofitable, and the outlook for asparagus culture was for a time very gloomy.

It was observed that some varieties, such as Argenteuil and Palmetto, were partially resistant. An organization was formed to develop a rust-resistant asparagus from numerous varieties of asparagus imported from foreign countries, and the breeding work was placed in charge of Mr. J. B. Norton. Among the imported varieties was one from England, named *Reading Giant*, which was more rust-resistant than any other. This was variable in type as well as in resistance, but selected individuals pollinated by selected male plants of this and other varieties have given rise to races of asparagus which are almost immune to rust and are at the same time of superior size, quality, and productiveness (1).

The problem of asparagus rust control was thus solved within five years. It remained only to disseminate the new resistant varieties.

[1] Invitation paper read before a joint meeting of the Botanical Society of America and the American Phytopathological Society at Pittsburgh, December 31, 1917.

[The **Journal** for May (5: 219-278) was issued June 21, 1918.]

Meanwhile a development of great biological significance was taking place. The old beds of susceptible American asparagus were killed out or plowed under as the weakened plants were no longer profitable, and replanting with Palmetto, Argenteuil, and Reading Giant was generally practiced throughout the eastern districts. At the same time the rust became less prevalent, except where infection centers of uncut, susceptible asparagus were permitted to remain. The elimination of the old non-resistant kinds is proving to be nearly as important as the introduction of the new resistant stocks.

We shall shortly be on the same basis as Europe, where asparagus and its rust parasite are both native, and where no serious losses occur since asparagus highly susceptible to rust has been eliminated. This fortunate result, which has taken place during our time and under our eyes, illustrates the fundamental principles which should guide our work with other crops, to the end that American agriculture may be protected against excessive losses from plant diseases.

Disease resistance in plants, as in animals, is nature's method of restricting parasites. Nature has been breeding disease-resistant plants since the world began. Evolutionary factors tend to modify the toxin formation of parasites and to build up the resistance of the host plants. Organisms react in this way upon each other throughout the range of their natural geographic distribution. The principal barriers are the seas, and we must, therefore lay much emphasis upon the *intercontinental* relations of this problem of disease resistance. The native parasites of our native plants, growing in their natural surroundings, offer no parallel to the ravages of introduced pests.

The most serious plant diseases are, in all countries, due to the bringing together of a host and a parasite native to different continents. When our forefathers cleared away the American forests and planted the European pears, the pears were ravaged by blight, *Bacillus amylovorus*, an endemic parasite of American pome fruits, which are, however, very resistant. Countless attempts to introduce the European grape, *Vitis vinifera*, into the eastern United States have failed because of the Phylloxera, black-rot and mildew, all native here; yet these diseases present a relatively slight obstacle to the culture of American species of grapes. The ravages of these diseases when carried from America to Europe nearly wiped out the viticulture of Europe, and they were checked mainly by the use of resistant American stocks.

The American gooseberry mildew, *Sphaerotheca mors-uvae*, is now repeating this history on the gooseberries of Europe. The late blight of potato and hollyhock rust are from South America, chestnut blight and citrus canker from Asia, white pine blister rust from Europe. In each case it is to be expected that resistant forms will be found to occur where the disease is native. This has in fact already been shown to be true, a recent and notable instance being that of the Chinese and Japanese chestnuts, which Dr. Van Fleet is using to cross with the American chestnut to produce a better and resistant tree.

The disease-resistance factor is important in all breeding. In some cases we may select directly for resistance from races of established quality. The wilt disease of cotton, due to the vascular parasite, *Fusarium vasinfectum*, occurs very commonly in the sandy soils of our cotton belt, from North Carolina to Texas, rendering cotton culture impossible on millions of acres. This disease has been overcome by the selection of resistant plants from fields where the disease had eliminated all others.

The cowpea suffers in the same area from a related parasite, *Fusarium tracheiphilum*, to which all varieties are subject except the Iron and its derivatives, Brabham and Monetta. That these cowpeas are also resistant to root-knot is most remarkable, considering that the nematode, *Heterodera radicicola*, has several hundred hosts, and that cowpeas, as a class, are very susceptible.

To produce a wilt-resistant watermelon it was necessary to hybridize with the hard-fleshed citron, and while good melons have been secured a fully satisfactory combination of resistance with quality and a rough rind for shipping has not yet been obtained (2).

Working to combat a related disease of cabbage, due to *Fusarium conglutinans*, Professor L. R. Jones has employed the same method of selection with great success. Cabbage yellows will no longer be feared when these new varieties are disseminated (3).

The Fusarium wilt of tomato, *Fusarium lycopersici*, a destructive disease in our central and southern states, is yielding rapidly before the plant breeder. The recent tests of F. J. Pritchard, of the Bureau of Plant Industry, show that a resistant late tomato has already been secured and that satisfactory early varieties are in sight.

The strains of flax resistant to *Fusarium lini*, bred by Bolley (4) and others make possible the continued culture of this important crop in regions where it was being abandoned because all the land was infested with the wilt parasite.

Equally remarkable are the results of James Johnson (5), who has selected strains of tobacco resistant to *Thielavia basicola*, a root parasite of many species and varieties of plants.

It is especially fortunate that resistant varieties may be employed against these root parasites which cannot be reached by fungicides or eliminated by rotation.

The problem now before us is to produce adapted races, resistant to disease, and bring them into general cultivation. There is reason to expect that when such a wheat as the Kanred, just described by Melchers, is in state-wide cultivation, stem rust will become insignificant in prevalence.

All plant breeding should take disease resistance into account. Strains under test should be exposed to infection by all the parasites that they are likely to meet in order to bring them into equilibrium, for it is possible by breeding plants in the presence of their diseases to produce resistant varieties. Such resistance will be reasonably permanent, at least as long as admixture and intercrossing with other non-resistant varieties are prevented.

There will, however, be serious danger that the advent of a new parasite, or even of a new biological strain, would result in losses. Such introductions, as we have seen, will be from other continents. Consequently, there is a fundamental biological argument for a policy of exclusion from North America of all living plant material from other continents, or at least for strict regulation and admission under safeguards as to disinfection.

International commerce has developed enormously in the last generation. Plant products may come from the ends of the earth; corn and potatoes have come from Australia, fruit from South Africa, and beans from Manchuria. These food products are to some extent a source of danger, since they may bring in new parasites; but the importation of nursery stock is a greater risk, for living plants are constantly accompanied by parasitic fungi and insects.

Complete success in excluding plant diseases is perhaps not to be hoped for, when one takes account of the myriad articles of commerce which may carry infection, such as wool, hides, and lumber; nevertheless, asparagus rust was not brought over until about 1896, chestnut blight not until after 1900, and citrus canker not until about 1911. That these diseases were kept out for so long when we had no exclusion law argues strongly for a hopeful view of the future.

If commerce in living plants is so dangerous, what view shall be taken of governmental plant introductions? The Office of Seed and Plant Introduction brings in seeds and plants from all over the world in small lots of a few seeds or buds each of thousands of species. If these introductions can be kept free from disease, and they have been thus far, they will be of invaluable assistance to the breeder. The great need is for a better organized use of this plant introduction service to bring in not only all the varieties of a crop but all its relatives that can be crossed with it, and, after propagation in a quarantine station, to place them at the disposal of breeders.

The improvement of our staple crops itself requires co-operative organization to conduct on a broader scale with ample material the work now attempted in an individual way. There is need of more systematic foreign exploration by specialists on the crops to be bred, who should in all cases be accompanied by pathologists to study the diseases that occur in the regions from which introductions are contemplated, and to assist in the collection of disease-free material. Foreign testing and propagation stations are also needed, the last to insure the sending of material free from insects and diseases.

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BEHAVIOR OF PLANTS IN UNVENTILATED CHAMBERS[1]

F. C. NEWCOMBE AND ETTA A. BOWERMAN

Within the past five years the writers have heard several botanists of good standing express their belief in the need of ventilation for plants, the notion being that plants in small chambers, in dark rooms, and even fungi in closed vessels, would grow and react better if the surrounding air was in motion.

This notion probably arose from the vagueness which, up to the last decade, existed as to the cause of "bad air" in rooms occupied by human beings, and the discovery, about ten years ago, that the ill effects of bad air could be removed by setting the air in motion. For over thirty years it has been known that bad air in unventilated rooms is not due to the accumulation of carbon dioxide. Various conjectures were offered as solutions of the problem. Perhaps it is not remarkable that some botanists should have imagined that their plants needed moving air for their well-being. This assumption of the need of moving air did not concern at all the oxygen or the carbon dioxide supply, but something else not more precisely defined.

Since, within the last decade, bad air in a room has been found in human hygiene to be due to excessive temperature and humidity, as the matter has been summarized by Hill, Flack, Rowlands, and Walker,[2] the effect of these conditions being manifested in the enlargement of the peripheral blood vessels and the consequent derangement in the heart-beat and the respiratory rhythm, it would seem difficult to apply the same causes to plants which have no circulation corresponding to that of the higher animals. Moreover, plants in a confined space almost never raise the temperature to the optimum for their growth; and, although they make the atmosphere very humid, humidity is generally favorable to their growth. But however improbable it may seem theoretically that experimental plants in confined spaces require ventilation, the plant physiologist needs to be assured

[1] Publication No. 168 from the Botanical Department of the University of Michigan.

[2] Hill, Flack, Rowlands, and Walker. Influence of Atmosphere on Health. Smithson. Misc. Coll. 60: 1. 1913.

by exact experiment. A narrative of such experimentation is given in the following pages.

EXPERIMENTATION

Growth of Plants in Unventilated Small Chambers

Series I.—The test for this series was made with plants confined under glass bell jars or under zinc cylinders closed at one end, bell jars and zinc cylinders having a capacity of 6 to 15 liters each, according to the size of the plants to be covered, and all placed in a darkroom of about 12 cubic meters capacity. For the standard, an equal number of plants of the same species were grown in uncovered pots in the same darkroom. An electric blower was employed to send day and night the air of the dark room in a gentle current over both sets of preparations. The temperature over the uncovered plants varied from 21° to 24° C.; in the bell jars and metal cylinders it was usually one degree higher by the thermometer, the difference in the showing of the thermometer being due probably to the current of air. The humidity over the uncovered seedlings varied from 35 percent to 58 percent; under the covers it stood near 100 percent.

The most of the work was done with seedlings, as seedlings are usually the plants employed for darkroom work. The seeds were planted in sphagnum moss in pots, and, as soon as the seedlings appeared above the moss, half of the pots were placed under the covers while the other half were exposed to the air current.

Observations and records were made every day through the period of the tests which varied with different sets from 5 to 17 days. The points for which observations were made were size of plants, time of unfolding of leaves, size of leaves, time of falling over of the seedlings, and general appearance.

The species and varieties of seedlings employed, with the numbers of plants, were as follows: *Zea mais* L. 140, *Zea mais* var. *everta* Sturtev. 54, *Raphanus sativus* L. 31, *Fagopyrum esculentum* Moench. 25, *Pisum sativum* L. 65, *Lupinus albus* L. 19.

Observation and measurements showed but slight differences between the plants raised under the two conditions. Members of the laboratory staff were asked to tell the differences between the two sets of plants when the pots were placed in two groups on a table, but were never able to name distinguishing features unless the comparison was made at a time near that of the exhaustion of the seedlings.

At such a time the seedlings from the confined air showed earlier exhaustion by falling over.

All species grow a little faster and reach their condition of exhaustion one to two days earlier in the small chambers. Measurements showed the Fagopyrum and the Lupinus attaining a slightly greater height in the current of air, while Zea, Raphanus, and Pisum grew higher under the bell jars. Only the Zea and Pisum produce, in the darkroom, leaves above the cotyledons. Measurements indicated slightly larger leaves from the quiet confined air.

Series II.—This series of tests was made in another darkroom of the same size as the preceding, and bell jars and zinc cylinders were used as before for one set of plants. Intermingled with the bell jars and cylinders stood the uncovered control plants, and a current of air, brought in from the adjoining greenhouse by a small blower and returned to the greenhouse, kept a constant draft around all the preparations. Except for the cooling effect of the greater transpiration from the exposed plants and the pots, the covered and uncovered plants must have been at the same temperature.

In general the seedlings used were planted in pots of earth, allowed to stand in the greenhouse till breaking through the ground, then placed under experiment in the darkroom. Some tests were made with seedlings which had reached a height of 10 to 20 cm. in the greenhouse before being placed in the darkroom. Since these latter showed in no way a behavior different from that of the younger seedlings, except that they did not live as long, they will not be given special consideration in the record.

A very thorough test was made with *Zea mais* L. (yellow dent), of which more than 400 seedlings were used in 7 distinct trials. The record for one of these trials with Zea is given here as representative for all:

"July 2, 1917. 30 seedlings about 10 cm. above earth in pots placed under zinc cylinders. 26 similar seedlings uncovered.

"July 9. All seedlings in each set have fallen over, none dead. Plants under cover, taller, thicker, and better looking than the uncovered. The 10 largest plants of the covered set average 47 cm. from ground to tip of longest leaf; 10 largest uncovered plants average 39 cm. Of covered plants, 13 have 3 leaves each; of uncovered, 7 plants have 3 leaves each. Many roots growing up into air from earth in covered pots; none in uncovered pots. Temperature has varied from 20° to 25°."

Besides the Zea of the last-described experiment, there were tested

by the same means 23 seedlings of *Pisum sativum* L., 26 seedlings of *Phaseolus vulgaris* L. (white kidney bean), 15 seedlings of *Phaseolus vulgaris* L. (black kidney bean), 55 seedlings of *Brassica alba* (L.) Boiss. Duration of the experiments was from 4 to 10 days.

The seedlings of Pisum and Phaseolus showed a greater height in the covered pots than in the uncovered, and, on the average, slightly larger leaves in the covered. The Brassica showed no differences under the two conditions, height of plants and size of cotyledons averaging the same. All species showed falling over about one day earlier in the covered pots. In the last days of some of the experiments some pots of seedlings under cover were attacked by damping off; the "falling over" mentioned is due to exhaustion of the plants, not to damping off.

Three species of larger leafy plants were subjected to the same test as the foregoing seedlings. The air in the darkroom being constantly brought in from the greenhouse in which the potted plants had been growing, there was no danger of the transposed plants suffering from a too dry atmosphere. The Ricinus plants used and the Bryophyllum were from 50 to 65 cm. tall, and cylinders correspondingly high were used to cover them. The experiments were made in July and August.

There were used 3 plants of *Ricinus communis* L. under zinc cylinders, and 6 plants uncovered. No differences in their growth or other behavior could be detected during the 10 days of trial. All began dropping their leaves after 7 days, and in 10 days from the beginning of the experiment all leaves had fallen.

Twelve plants of *Bryophyllum calycinum* Salisb. were put under experiment, 6 for 16 days and 6 for 27 days; in each experiment, 3 plants were kept under zinc cylinders and 3 were left u[illegible]vered and thus exposed to the moving air. No differences in behavior could be seen. The new leaves unfolded in the dark were pale yellow, but no leaves fell from either the covered or uncovered plants.

Growth of Plants in Ventilated and Unventilated Chambers of Equal Size

Series III.—The chambers used in this series were two wooden culture boxes, 20 x 20 x 50 cm. Each box had a closely fitting rabbeted door. In each box were placed three 4-inch pots of seedlings of *Brassica alba* (L.) Boiss. about 4 cm. tall, growing from earth. These boxes were placed in an interior darkroom with a capacity of

12 cubic meters, the air in which was constantly agitated by an electric fan. One box had its door ajar 5 cm., the other was tightly closed. The seedlings in each box numbered 60. The temperature ranged from 23° to 25°. The test lasted for 4 days.

During the progress of the experiment, it was necessary to water the plants in the open box twice. The other box was not opened during the 4 days, and the earth was moist at the close of the experiment.

Except for a little more damping off in the closed box (there was some in the open box), no differences could be seen in the two sets of plants at the end of the 4 days. The general thriftiness of the seedlings from the closed box seemed a little greater than that of the seedlings from the open box. Measurements showed no difference in the average height of the two sets of plants.

Series IV.—A very satisfactory series of experiments was carried through by employing two large wooden boxes, keeping the air stagnant in one and in movement in the other, the two boxes sitting side by side in a quiet corner of a room with thermostatic control. Each box was 35 x 60 x 90 cm., was carefully lined with heavy paper to reduce the exchange of air with the outside, and had a closely fitting cover. To secure agitation of the air in one box, a small blower was set outside, whose inlet and outlet were connected with apertures in the box by short lengths of iron pipe 10 cm. wide. It was soon found that the motor of the blower heated the air passing through the blower 1° to 2° higher than the air in the other box. The difficulty was overcome by covering a portion of the blower pipe with absorbent paper and causing water to drip on the paper. After some adjustments had been made, the air in the two boxes, as shown by inserted incubator thermometers, kept fairly well the same temperature, never differing in the two boxes more than 0.4° C. Temperatures during the experiment ranged from 18° to 21.5°. Observations were made every 2 or 3 days, and the tests lasted from 7 to 19 days.

The following seedlings were tested, growing in pots of earth; the experiments being started as soon as the seedlings had broken through the ground: *Zea mais everta* Sturtev. 140 plants, *Zea mais* L. (yellow dent) 41, *Triticum vulgare* Vill. 39, *Lupinus albus* L. 158, *Pisum sativum* L. 38, *Lathyrus odoratus* L. 120, *Vicia faba* M. (Windsor broad) 37, *Ricinus communis* L. 12.

The difference in humidity in the two boxes was considerable,

though much smaller than in the previous comparisons. This reduction of the difference in humidity is followed by the nearly complete disappearance of the greater height attained by plants under the bell jars.

In some of the sets with Zea, seedlings in the box with still air grew thicker and taller; the next week, with a like test, results were exactly reversed. The same reversal occurred with Lathyrus. Lupinus and Vicia gave a little greater average in height and thickness of seedlings in quiet air, Ricinus in moving air, while measurements could detect no difference between the two cultures of Triticum. Lathyrus and both varieties of Zea showed more individuals dying in 18 days in moving than in quiet air, while, of the other species used, few individuals died and there was no larger percentage of deaths in one box than in the other. Persistent differences in the time of unfolding of leaves in quiet and in moving air could not be established.

Using the same two boxes as with the foregoing seedlings, 6 potted plants of Coleus were employed, 3 in each box. The plants were each about 10 cm. high, each with 4 pairs of mature leaves, and all growing well when they were taken from the greenhouse. To make the moisture conditions more nearly equal in the two boxes, four basins, 20 cm. in diameter, were filled with water and set in the box whose air was to be agitated by the blower.

The 6 plants were kept in the dark boxes for 10 days, temperature in the two boxes not differing more than 0.4° C., and the temperature of the room containing the boxes ranging from 18° to 21.5° C. Examination and measurements were made every 2 or 3 days. That the pots in the moving air were still in a drier atmosphere than those in quiet air was shown by the more frequent watering needed by those in moving air. The effects noted in the foregoing cases were still apparent here, though to a very slight degree. At the conclusion of the 10-day period, the 3 plants in moving air showed a total elongation of 13.7 mm., and had dropped 12 leaves. The 3 plants in quiet air showed a total elongation of 14.7 mm., and had dropped 10 leaves. Other differences were not apparent.

Series V.—This series of experiments was carried out in two constant temperature dark cabinets of about 12 cubic meters capacity each, in one of which an electric fan was kept in constant motion, while in the other the air was quiet. The temperature for both cabinets was regulated for 23°, with an extreme variation of 1° C.

Zea mais (yellow dent) seedlings were set in these cabinets just as the plants were breaking through the earth. In the agitated air were 16 seedlings, in quiet air 17 seedlings. After 6 days, 4 plants in each cabinet had fallen over. The plants in quiet air averaged a little taller than those in moving air. No other differences could be detected. Ten days after the beginning of the test, all plants in moving air had fallen over, and all but one in quiet air had fallen over. The average height of the 16 plants in moving air was 27 cm.; of the 17 in quiet air, 29 cm. Of the plants in moving air, 5 had 3 leaves and 11 had 2 leaves each; of those in quiet air, 5 had 3 leaves and 12 had 2 leaves each. No other differences could be seen between the two sets of plants.

For the sake of comparison with the two foregoing groups of plants, a third set of 15 Zeas was run at the same time under small zinc cylinders in one of the cabinets. At the end of the 6-day period, 11 of the 15 had fallen over, compared with 4 in each of the other sets. At the end of the 10 days, all 15 plants had fallen over. The average height of these was 37 cm. compared with 27 and 29 cm. respectively in the other two groups; and of the plants under the zinc covers, 10 had 3 leaves and 5 had 2 leaves, there being thus twice as many with 3 leaves each as in each of the other two groups. These comparative results bring out strongly the effect of excessive moisture induced by a small confined space.

None of the falling of the plants noted above was due to damping off.

Fagopyrum esculentum seedlings were placed in the dark cabinets, 4 pots in each, with 10 seedlings in each pot, seedlings grown in the greenhouse to a height of 2 to 5 cm. The temperature was 23° C. in each cabinet, the air agitated in one, quiet in the other.

After 6 days, 29 of the 40 seedlings in quiet air and 33 of the 40 in moving air had fallen over. Thus there were 7 erect in moving air and 11 erect in quiet air. The number of dead seedlings was about the same in each group. As the experiment progressed during the 6 days, no certain difference in general behavior could be observed. The small difference noted above is probably not significant; another test might reverse relations.

Lupinus albus seeds were planted in whitewood (Liriodendron) sawdust, from which 38 seedlings grew in one pot and 46 in the other. When the first seedlings appeared above the sawdust, the pots were removed to the two dark cabinets, one pot in moving, the other in

quiet air. The growth and general behavior were followed for the ensuing 10 days, but no differences could be detected. The seedlings began falling over from weakness 7 days after they had been placed in the cabinets. At this time, the largest seedlings in each pot were 16 to 19 cm. high. Of the 38 seedlings in moving air, 9 showed primary leaves emerging between the half-open cotyledons; while among the 46 seedlings in quiet air, 11 showed primary leaves emerging between the opening cotyledons. At the end of 10 days in the dark cabinets, the most of the seedlings in each pot had fallen over. None were dead, and there was no damping off.

The temperature during the experiment had ranged from 22° to 23°.

Brassica alba seedlings, to the number of 72 in moving air and 63 in quiet air, were grown in pots of earth, the preparations being placed in the two dark cabinets before the seedlings had broken through the ground. The plants grew in the two cabinets without showing noticeable differences, except that the cotyledons opened a little earlier in the quiet air; on the fifth day of the experiment, 12 seedlings in moving air and 16 in quiet air showed expanded cotyledons. On the sixth day, all but 8 seedlings in moving air, and all but 19 in quiet air had fallen over. The 10 longest hypocotyls in moving air averaged 15 cm.; in quiet air, the 10 longest averaged 14.5 cm. There were no cases of damping off.

The temperature had ranged for the 6 days from 22° to 23°, and kept the same in both cabinets.

Cucurbita pepo L., with seedlings to the number of 63, gave the same general result as the foregoing species. Differences in behavior in moving and in quiet air in the cabinets could not be discerned.

Sensitive Reactions in Ventilated and Unventilated Chambers

Besides the criteria of the amount of growth, unfolding of leaves, and the vital period, used in the foregoing pages to estimate the effect of ventilation, it would be worth while to employ sensitive reactions also as a criterion. For this purpose geotropism and heliotropism have been studied with the plants in two small culture boxes, the one ventilated, the other closed against the exchange of air. These boxes were of wood 20 x 20 x 50 cm., with a closely fitting rabbeted door, and an aperture in one end 5 x 10 cm. The boxes stood side by side in a darkroom in which an electric fan was kept in constant motion.

The temperature of the darkroom was kept at 24° to 25° C. The aperture in the end of one box was tightly closed by a glass plate held in position by a border of heavy paper pasted to the glass and the wall of the box. The fan was placed so that a gentle current of air flowed obliquely against the aperture end of each box.

Geotropism.—Three 4-inch pots of seedlings of *Brassica alba*, 20 seedlings in each pot, the seedlings raised in the same darkroom as that in which the later experiment was made, and having a height above the earth of 2.5 to 5 cm., were placed in each culture box in the erect position, and so left for 18 hours. Without opening, the boxes were now turned so that the included pots were brought into the horizontal position, the pots having previously been secured to prevent rolling.

Observation at the end of two and one half hours showed in the ventilated box 50 of the 60 plants with geotropic angles of 15° to 90°; in the closed box, 48 of the 60 showed corresponding curves. The angles seemed to average a few degrees more in the closed box, but this was only an estimate. No accurate measurements were made.

Six and one half hours after turning the plants to the horizontal position, all the seedlings in the ventilated box and all but one in the closed box showed negative geotropic curves of 20° to 90°. A greater average angle for either set of plants could not be determined.

Heliotropism.—Six 4-inch pots of seedlings of *Brassica alba* were raised in the greenhouse to a height of 2 to 3 cm. above the earth, then transferred to the dark cabinet last used, and 3 pots enclosed within the closed culture box used in the preceding experiment, while the other 3 were placed in the ventilated culture box. In this condition the 6 pots remained in the dark for 18 hours, the electric fan keeping the air of the cabinet in circulation. In order that the plants in both culture boxes might have the same amount of light in the subsequent stimulation, a glass plate, like that used in closing one of the culture boxes, was placed inside the other box and 4 cm. distant from the aperture, so that all light which reached the plants had to pass through similar glass in each box, but the current of air could enter one box and not the other.

A single tungsten lamp of 25 watts was used for both boxes, the boxes being set with their long axes pointing toward the lamp. The middle of the pots in each box were respectively 25, 38, and 51 cm. distant from the lamp. The temperature was 24° C.

After the preparations had been in the dark for 18 hours, the light was turned on. Observation after one hour showed the seedlings in each box in the pot nearest the light responding with positive curves. The responses seemed about equal in the two boxes. There were a total of 62 seedlings in the ventilated box, and 71 in the unventilated box. Two hours after the light was turned on, the pots in the ventilated box, arranged in the order of their nearness to the light, showed respectively 95 percent, 73 percent, and 21 percent of the seedlings with positive angles of 20° to 60°. Those in the unventilated box, in the same order, showed 100 percent, 48 percent and 45 percent with positive angles of 20° to 60°. The average angle seemed about the same in both boxes.

Six hours after the illumination began, in the ventilated box 12 of the 62 seedlings were still erect. In the unventilated box, 7 of the 71 seedlings were still erect. This difference is not significant. The experiment ends with no evidence tending to show that confinement in a small unventilated chamber retards heliotropic response.

The foregoing test was repeated with 3 pots of seedlings of *Brassica alba*, and 3 pots of seedlings of *Fagopyrum esculentum* in the ventilated box, and the same number of pots of each species in the unventilated box. At the same time 3 pots of each species were put into an adjoining cabinet of equal size (12 cubic meters), where the temperature remained the same as in the first cabinet, and where the air was quiet. The 6 pots last mentioned were not put into a culture box.

Early observation for incipient heliotropic curvature was not made; but, after the illumination had continued for 12 hours, all plants had responded, and no differences in the behavior of the 3 groups of seedlings could be detected.

Summary and Conclusions

The work narrated in the present paper was undertaken to determine whether plants behave as well in quiet air in confined chambers as in moving air or in ventilated chambers. The question is of practical importance in experimental work chiefly in the growth of plants in dark boxes and darkrooms. The growth of plants in stagnant air in the light involves several other conditions, and no experiments in the light were undertaken.

The material employed was mostly seedlings, of which about 2,000 have been used, belonging to 12 species. Larger plants belonging to 3 species were also used.

The chambers for securing stagnant air have varied in size from bell jars of about 6 liters capacity to darkrooms of 12 cubic meters. While one set of plants was growing in the chambers in quiet air, another control set was always growing in a chamber of equal or larger size whose air was stirred constantly with a fan or with a blower.

The criteria for comparing behavior in quiet and moving air were duration of vital period in the dark, size of seedlings, size and number of leaves formed, general vigor of the plants, phenomena of geotropic and heliotropic reaction.

It may be said in a word that no ill effects manifested themselves as due to confining plants in stagnant air in small or in large chambers. The greatest objection to small chambers, as bell jars, comes from the growth of injurious fungi on the plants, due to the excessive moisture. This ill effect mostly disappears when the plants are kept in a larger chamber, or when care is taken to keep the uppermost stratum of the soil free from excessive moisture.

With but few exceptional cases, the cultures have shown that in small chambers, like bell jars, seedlings grow taller, produce larger and more numerous leaves, and become exhausted so that they fall over a day or two earlier (at 20°) than in a larger ventilated chamber. These differences were pronounced when comparing plants grown under bell jars with similar plants grown in moving air, the moving air coming either from the outside or being circulated within the darkroom. The differences were much less when the chamber with stagnant air was increased to a fifth of a cubic meter, and disappeared in most cases when the two chambers for comparison were 12 cubic meters each, the one with stagnant, the other with circulating air.

This larger and more rapid growth in very moist air is no new discovery. It has been cited by Wiesner[3] and by Eberhardt.[4]

Not only is ventilation of no effect in producing better seedlings in a small or large chamber in the dark, but it has no visible effect on the sensitive reactions of geotropism and heliotropism.

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[3] Wiesner. Formänderungen von Pflanzen bei Cultur im absolut feuchten Raume und im Dunkeln. Ber. Deutsch. Bot. Ges. 9: 46. 1891.

[4] Eberhardt. Action de l'air humide sur les végétaux. Compt. Rend. Acad. Sci. (Paris) 131: 193. 1900.

SEGREGATION OF SUSCEPTIBILITY TO PARASITISM IN MAIZE

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Various degrees of immunity, shown by cultivated plants to the attacks of parasitic organisms, have been recorded. Resistance of cotton, cowpeas, and melons to fungus injury has been reported by Orton (1, 2, 3), of potatoes by Stuart (4), and of flax by Bolley (5). Webber and Orton (6) have shown that the same variety of cowpeas which is resistant to wilt is also markedly resistant to the root knot nematode. Gernert (7) also states that teosinte and first generation teosinte-maize hybrids are not subject to injury by aphids which attack maize.

The inbred strains of maize carried on at the Connecticut Experiment Station, reported by East and Hayes (8), which have been continuously selfed to the present time, show striking differences in the number of plants affected by the smut fungus (*Ustilago zeae* (Beck.) Ung.) and an unidentified leaf blight organism. In 1915 a few inbred strains of flint maize were badly injured by this leaf blight organism. Again in 1916 the same strains were affected in the same way, every plant being badly injured, while other selfed strains derived from different varieties and grown in adjoining rows were almost entirely unaffected. First generation hybrids of these susceptible strains with resistant types were grown in the same field and were only slightly damaged.

In 1917 no injurious effects of this nature could be seen on any plants. Several different strains, however, were badly attacked by smut. In table 1 are given the number of plants grown and the number of plants affected of sixteen different selfed strains of maize, inbred from nine to eleven generations. The relation of these to each other is shown by the pedigree numbers. Twelve of these were derived from one original variety. Of these, three strains were started from single plants in the first generation of inbreeding and numbered 1–6, 1–7, and 1–9. In the third generation from 1–7 two lines were started, 1–7–1–1 and 1–7–1–2, and continued. After the seventh generation

all lines were again split up, but at that time the plants were nearing complete homozygosity and the lines which were separated after the

TABLE 1

Susceptibility of Inbred Strains of Maize to Smut (Ustilago zeae)

Pedigree Number	Plot I			Plot II			Plot III		
	Number of Plants Grown	Number of Plants Affected	Percent Affected	Number of Plants Grown	Number of Plants Affected	Percent Affected	Number of Plants Grown	Number of Plants Affected	Percent Affected
10-4-8-3-5-3-4-8-2	29	0	0	227	0	0	—	—	—
10-4-8-3-5-3-4-5-2	21	1	4.76	223	6	2.69	—	—	—
10-3-7-3-9-7-5-1-1	20	0	0	242	3	1.24	113	0	0
10-3-7-3-9-7-5-4-2	25	0	0	274	2	.73	—	—	—
1-9-1-2-4-6-7-5-6-2	31	0	0	183	0	0	84	0	0
1-9-1-2-4-6-7-5-3-2	17	0	0	185	1	.54	96	1	1.04
1-7-1-2-2-9-2-1-1-1-1	27	0	0	86	0	0	—	—	—
1-7-1-2-2-9-2-1-1-4-3	19	1	5.26	196	1	.51	80	0	0
1-7-1-1-1-4-7-5-2-1-1	29	0	0	224	18	8.04	—	—	—
1-7-1-1-1-4-7-5-2-6-1	16	2	12.50	157	15	9.55	—	—	—
1-7-1-1-1-4-7-5-4-7-1	21	3	14.29	211	28	13.27	52	3	5.77
1-7-1-1-1-4-7-5-4-5-2	25	3	12.00	215	21	9.77	—	—	—
1-6-1-3-4-4-4-2-5-5-2	20	0	0	183	0	0	26	0	0
1-6-1-3-4-4-4-2-5-3-2	27	0	0	195	0	0	—	—	—
1-6-1-3-4-4-4-2-4-1-3	30	0	0	211	0	0	—	—	—
1-6-1-3-4-4-4-2-4-4-2	20	0	0	200	0	0	80	0	0

seventh generation remained quite similar. So that for our purpose four distinct lines descending from one original variety and two lines from a different variety can be considered.

These strains were grown in duplicate and some in triplicate plots in three rather widely separated parts of the same field in 1917. Since the same strains show about an equal amount of susceptibility or immunity in all three plots, it seems evident that the pathological

TABLE 2

Summary of Figures Given in Table 1

Pedigree Number	Percent of Plants Affected				Total Number of Plants Grown
	Plot I	Plot II	Plot III	Total	
10-4-etc.	2.00	1.33	—	1.40	500
10-3-etc.	0	.97	0	.74	674
1-9-1-2-etc.	0	.27	.56	.34	596
1-7-1-2-etc.	2.17	.35	0	.49	408
1-7-1-1-etc.	8.79	10.16	5.77	9.79	950
1-6-1-3-etc.	0	0	0	0	992

differences are not due to differences in local infection. Maize was grown on the field the previous year and smut was fairly abundant, so that there was probably as good an opportunity for the plants to become infected in one place as in another. Although smut infection is not considered to come from the seed in the case of maize, the possibility of this factor as a cause of the similar results in the different plots is ruled out because the seed for any one strain grown in the three plots came from different hand-pollinated plants grown the previous year. So that if one strain was badly affected in one plot the fact that it was also affected in another plot was not due to the use of the same contaminated seed. The seed plants of all the different strains were grown in the same place in 1916 and had an equal opportunity of infection.

No data were taken on the infection by smut previous to 1917. It is quite likely that these strains will show variability in the extent to which they are infected by smut in different years, but, as shown in table 1 and summarized in table 2, out of nearly one thousand individuals not a single plant of strain number 1–6–1–3 was affected, while in a nearly equal number of plants of strain number 1–7–1–1 about

TABLE 3

Susceptibility to Smut of a Non-inbred Variety of Maize, of Two Inbred Strains Derived from this Variety, and of the First and Second Generation Hybrids between These Two Inbred Strains

Pedigree Number	Number of Plants Grown	Number of Plants Affected	Percent of Plants Affected
1	114	2	1.75
1–7–1–1–4–7–5–4–7–1	52	3	5.77
1–6–1–3–4–4–4–2–4–4–2	80	0	0
{(1–7–1–1–4–7–5–4–7) × (1–6–1–3–4–4–4–2–4–4)} F_1	36	0	0
{(1–7–1–1–4–7–5–4–7) × (1–6–1–3–4–4–4–2–4–4)} F_2	97	5	5.15

ten percent of the plants were affected. These two strains were grown in rows side by side in three different plots. The other two strains derived from this same source and the two strains from another variety all show a small amount of infection. Clearly there are marked differences in susceptibility in these strains. The differences are reasonably consistent in the three different places in which the plants were grown and show without a doubt that segregation of susceptibility to

infection by the smut fungus has taken place during the reduction to homozygosity accompanying the inbreeding process.

Strain number 1–6–1–3, which is not at all affected, is the most vigorous of the four inbred lines derived from variety number 1. It has a darker green color, the plants are larger and more productive than any of the other three. In table 3 the data on smut infection of the first and second generation hybrids of this most resistant strain with the most susceptible strain are given, together with the figures for the two parents and the original non-inbred variety from which the inbred strains were derived. All of these were grown in five adjacent rows in plot III. Owing to the poor germination of the seeds of one of the parents and the first generation of the cross, the numbers are too small to put much reliance upon. Smut was not shown by any plants of the immune parent and of the first generation hybrid, but appeared on some of the plants of the original variety, the susceptible parent, and the second generation of the cross.

The numbers given in table 3 represent all the plants that were grown of the original variety and the second generation hybrid. Of the first generation hybrid, 439 plants in all were grown in different parts of the field. Of these, 2.28 percent were affected, showing that the dominance of immunity is not perfect. When this figure is compared with the 9.79 percent of affected plants of one of the parents, it shows clearly that the hybrid approaches the condition of the immune parent.

As in so many other cases, those factors which enable an organism to attain the best development tend to dominate. The facts given here are considered as additional support to the hypothesis advanced by the writer (9, 10) that the increase in development commonly shown by hybrids is due to the conjunctive action of a large number of favorable, dominant growth factors contributed by both parents.

Resistance is not shown by all first generation hybrids. Biffen (11) finds that some first generation wheat crosses are susceptible to rust and Norton (12) that certain tomato hybrids are susceptible to a wilt disease. On the other hand Vavilov (13) reports wheat hybrids resistant to mildew and Stuckey (14), tomatoes which are resistant to blossom-end rot in the first generations. All these crosses were between types which differed in their susceptibility. Rasmuson (15) with reference to the grape Phylloxera; Van Fleet (16), the chestnut blight; Orton, the melon wilt; Blinn (17), the cantaloupe leaf blight;

Jesse B. Norton (18), the asparagus rust; L. R. Jones (19), the flax wilt; and Gernert with reference to the corn aphis, noted above, all find that resistance tends to be shown in the first generation of crosses between types which are classed as resistant and susceptible.

Although resistance is a highly complex and variable character there can be no doubt but that many types of resistance are determined by definitely inherited factors more or less independent of the environment and of heterosis. There may be certain general principles involved in that certain types of disease thrive best in plants which are in a luxuriant condition either from environmental causes or from the vigor of hybridization. If that were the case resistance would tend to be recessive in those plants which show heterosis markedly. On the other hand certain other infections may be more pronounced in less vigorous plants. In that case heterosis would tend to keep the first generation plants free from the attacks so that the resistance would seem to be dominant.

It is doubtful, however, that in the case of the susceptibility of maize to the smut fungus, reported here, vigor can be more than a minor variating factor. Although the most resistant strain (no. 1-6-1-3) is the most vigorous of the four inbred lines, many naturally crossed varieties are several times as vigorous and productive and are quite susceptible. And there are other inbred strains (nos. 1-9-1-2 and 1-7-1-2) which are no more vigorous than the most susceptible one (no. 1-7-1-1) yet are only slightly susceptible.

The evidence from these inbred strains of maize and their first and second generation hybrids seems conclusive that susceptibility is governed by factors which are capable of being segregated into some lines and not into others and that the modification of the expression of the parasitism by the vigor of the plants is a minor consideration.

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THE VALUE OF CERTAIN NUTRITIVE ELEMENTS IN THE DEVELOPMENT OF THE OAT PLANT

JAMES GEERE DICKSON

INTRODUCTION

It has long been recognized that the incombustible residue resulting from the incineration of a plant is of great importance, and a large amount of work has been done to determine the chemical combinations resulting from the incineration, since by this means it was hoped to determine the nutritive elements which are essential to the plant. This method has proven only partially successful. Liebig (1855), basing his conclusions upon water culture methods, advanced the view that certain salts are indispensable to plant development and maintained that the productiveness of the soil is determined by the essential salts present. Liebig's hypothesis stimulated research, and it was later determined that six ash constituents are essential to the growth of phanerogams, viz., calcium, magnesium, potassium, sulphur, phosphorus, and iron, to which should be added nitrogen, a constituent not found in the ash.

Many similar experiments confirmed these results, but the general trend of investigation soon changed to the study of the function of the elements thus shown to be essential. Various writers, more recently Chirikov (1914) and Truog (1916), have attributed to calcium the function of acting as a carrier of the essential phosphoric acid and as a neutralizer of the organic acids formed in protein synthesis. In agreement with several early workers, Hansteen (1910) has described calcium as functioning in the transformation and transfer of carbohydrates and in the formation of cell walls by green plants. On the other hand, Robert (1911, 1912), confirming the results of earlier investigators, has shown that calcium is not an essential element in the nutrition of fungi, and Molisch (1895) has found that certain algae can thrive without calcium. The effect of calcium in reducing the toxic action of other bases, notably magnesium, in the culture solution has been studied especially by Loeb (1906), Osterhout (1906*a*, 1908, 1911, 1912, 1916), and McCool (1913).

Boehm (1875) was perhaps the first to call attention to some of the specific actions of magnesium salts in culture solutions, and much recent work has demonstrated the toxic action, at least upon green plants, of magnesium salts either alone or when present in excess of a certain ratio to the other bases. It has been shown that magnesium is required for the proper growth of fungi and that, contrary to what occurs in the case of green plants, magnesium may be present in very large quantities without causing a toxic effect. These results have been confirmed by the unpublished work of Mr. J. P. Bennett in this laboratory. This relation of magnesium is in part explained by the fact that magnesium neutralizes the organic acids formed in certain metabolic processes of the fungi and is thus taken out of the field of action. Loew (1892), Bokorny (1895), and Reed (1906) have suggested that magnesium plays an important part in the assimilation of phosphorus and phosphoric acid. It has been found by Sullivan (1905) that certain bacteria can form lipochromes in normal quantity only in the presence of magnesium sulphate and of a phosphate. Others have demonstrated the intimate relation between the presence of magnesium and the formation of vegetable oils, which probably accounts for the high magnesium content of many seeds. Aso (1901) had previously shown by analyses that spores of *Aspergillus oryzae*, which contain a large amount of fat, contain also a moderately large proportion of magnesium.

Nobbe (1870) first pointed out that carbohydrates are formed normally only in the presence of potassium, and Loew (1880) suggested that potassium plays an important rôle in promoting the chemical condensation of proteins, carbohydrates, and fats in plant synthetic processes. Loew's idea is partly based upon Nägeli's (1879) assertion that the potassium salts are better adapted to catalytic work than sodium salts, because of their generally greater affinity for water. It was shown by Schimper (1890) that potassium is essential to the normal development of growing apices. Breazeale (1906) found that plants previously grown in solutions lacking potassium or sodium absorb large quantities of potassium when transferred to a normal nutrient solution containing both these elements.

Early investigations demonstrated that phosphorus is necessary for plant growth and that it occurs chemically combined with many plant substances. Harden (1911) suggested the necessity of phosphorus in fermentation and other enzymatic activities.

More recently thorough studies on the physico-chemical relations existing between the various compounds and elements in culture solutions have been made in an attempt to understand some of the factors neglected in the earlier work. Livingston (1906), Breazeale (1905), Osterhout (1906*b*), Tottingham (1914), and Shive (1915*a*, *b*) have contributed to our knowledge of balanced nutrient solutions.

It has been the purpose of the experiments herein recorded to study the effect of some of the essential nutrient elements upon the development and composition of plants when other physico-chemical factors, such as unequal osmotic pressure and the addition of new chemical elements, were controlled as far as possible.

The culture work from May to August, 1915, and May to August, 1916, was carried on under climatic conditions different from those that governed the latter part of the work, which differences may in some cases explain the differences between the data recorded for the two respective periods. In a later paper a comparison will be made of the meteorological conditions for the two periods.

From September, 1916, to date the work has been carried on in the laboratory of plant physiology at the University of Wisconsin. It is a pleasure to acknowledge my indebtedness to Professor James Bertram Overton for his advice and assistance in supplying the rather extensive apparatus necessary during the course of this investigation.

Methods

Knop's standard nutrient solution, slightly modified by adding 0.1 gram of sodium chloride per liter, which made a salt concentration of 1.5 grams per liter instead of 1.4 grams, was used as the standard nutrient solution throughout the series of experiments. Standard laboratory chemicals were used during the first two years, but on account of the large proportion of impurities, which caused considerable difficulty in the physical study of the solutions and which might also be expected to produce important modifications in the results of the experiments, it was thought best to use the purest chemicals obtainable for all later work. Kahlbaum's analyzed chemicals were finally used, with the exception of potassium nitrate and calcium chloride, of which Merck's "Blue Label" brand was used, and with the exception also of calcium nitrate, which was prepared from a special grade of nitric acid and Iceland spar ($CaCO_3$). All chemicals

were reanalyzed for the elements related to the work and calculations were based upon these analyses.

The chemicals were dried at constant temperature until anhydrous or until a definite amount of water remained and then reweighed; distilled water was then added to give the required salt concentration for the stock solutions. In the preparation of the salts it was necessary to make extensive determinations of water content and composition at the various temperatures used in those cases in which no published data for the compound in question could be found. Whenever the methods of other investigators were used they were carefully checked in all cases before being adopted as standards for drying the salts.

Calcium nitrate, potassium nitrate, monopotassium phosphate (Tottingham, 1914), sodium chloride, sodium nitrate, and potassium chloride were dried at 150° C., and weighed as anhydrous. Magnesium sulphate was dried at 160° and treated as containing one molecule of water (Tottingham, 1914). Sodium sulphate was dried at 200° and weighed as anhydrous. Calcium chloride was dried at 200° and treated as containing 0.2 percent water by weight (Weber, 1882). Magnesium nitrate was dried over commercial sulphuric acid (sp. gr. 1.84) and treated as containing two molecules of water. Monosodium phosphate was dried at 100° and weighed as anhydrous. Ferric chloride was weighed without previous drying and considered as containing six molecules of water.

The modified Knop's solution which was used in all the work, as shown in table 1, was of one tenth the concentration described above, and contained, therefore, 0.15 grams total salts per liter instead of 1.5 grams. Previous work has shown that, when wheat and oats are watered during the growing season with the nutrient solution at the higher concentration, the accumulation of salts exerts an inhibiting influence upon their growth.

2.5 cc. of a $M/100$ solution of ferric chloride were added to every liter of culture solution.

The complete cultural series consisted of the "normal" solution, that is, of Knop's solution modified as shown in columns three and four of table 1, and five further modified culture solutions in each of which one of the elements magnesium, calcium, potassium, phosphorus, and nitrogen was reduced to one tenth of the quantity present in the "normal" solution (tables 2 and 3). A quantity of each solution sufficient for the demands of the entire growing season was made up

from the stock solutions diluted with water condensed from a block-tin still. The average conductivity of the water at a carbon-dioxide equilibrium with the air was 0.9×10^{-5} reciprocal ohms. The solutions were stored in fifty-liter pure flint glass containers, from which they were drawn when needed.

TABLE I

The Composition of Knop's Nutritive Solution and of the Modified Knop's Solution Used as the Normal Culture Solution.

Salt	Grams per Liter of Solution		Concentration of Salts Used in Modified Solution	
	Standard Knop's	Modified Knop's	Grams per Liter of Solution	Percentage Composition
$Ca(NO_3)_2$	0.8	0.8	0.08	0.0533
KNO_3	0.2	0.2	0.02	0.0133
KH_2PO_4	0.2	0.2	0.02	0.0133
$MgSO_4$	0.2	0.2	0.02	0.0133
NaCl		0.1	0.01	0.0067
Total salt	1.4	1.5	0.15	0.1000

DISCUSSION OF THE NUTRIENT SOLUTIONS IN TERMS OF CONCENTRATION

Throughout the following discussion concentration will be expressed in terms of percentage of dissolved salts, that is, of grams per 100 cc. of solution, and also in osmotic concentration. The osmotic concentration was calculated upon the basis of electrolytic dissociation as given by Jones (1911, 1912) for all the salts employed except monopotassium phosphate and monosodium phosphate, the data for which are not given in Jones' tables. The electrolytic dissociation of monopotassium phosphate was taken from Tottingham's (1914) table; that of monosodium phosphate was determined by the writer. All measurements were taken at 25° C., which approximates the average temperature of the culture solutions during the growing period. The procedure, as fully described by Tottingham (1914), is based upon the fact that osmotic pressure is a colligative or additive property, depending upon the total number of particles (ions, molecules, and molecular aggregates) in the solution irrespecitve of their kind. An approximation to the total osmotic concentration of any solution may be obtained by summing the values of the partial concentrations of the constituent salts (tables 2 and 3).

TABLE 2

The Composition of the Normal Solution in Terms of Osmotic Concentration

Salt	Dissociation Factor	Concentration			
		Percent	Volume Molecular		
			Decimal	Fraction	Osmotic
$Ca(NO_3)_2$	1.94	0.0533	0.00325	$M/$ 307.7	0.0063
KNO_3	1.90	0.0133	0.00132	$M/$ 758.4	0.0025
KH_2PO_4	1.86	0.0133	0.00098	$M/$1021.8	0.0018
$MgSO_4$	1.87	0.0133	0.00111	$M/$ 903.1	0.0022
NaCl	2.00	0.0067	0.00114	$M/$ 872.5	0.0023
Total salts		0.1000	0.00780	$M/$ 128.2	0.0151

The dissociation of the salts, with the exception of monopotassium phosphate and monosodium phosphate, is considered as occurring in one step in each case as illustrated by the following equations:

$$KNO_3 = K + NO_3,$$
$$MgSO_4 = Mg + SO_4.$$

The two phosphate salts are considered as dissociating in four steps instead of one as shown in the following scheme, which illustrates the complexity of electrolytic dissociation in a solution of monopotassium phosphate, when the process is incomplete and proceeds by stages with increasing dilution.

Stage I. KH_2PO_4.
Stage II. (1) KH_2PO_4, (2) K, (3) H_2PO_4.
Stage III. (1) KH_2PO_4, (2) K, (3) H_2PO_4, (4) H, (5) HPO_4.
Stage IV. (1) KH_2PO_4, (2) K, (3) H_2PO_4, (4) H, (5) HPO_4, (6) H, (7) PO_4.

The algebraical method of calculation of the dissociation of these salts is explained in detail by Tottingham (1914). For practical purposes only the first two dissociation steps indicated above need be considered for monopotassium or monosodium phosphate.

The effects of dissociation in a complex mixture of salts, even when very dilute, are not easily determined. In such a mixture, the influence of one salt upon the dissociation of another should be taken into account in work of this character, but the problem becomes complicated by the difficulties attending calculations. It was not

TABLE 3

The Composition of the Modified Solutions in Terms of Osmotic Concentration

Salt	Dissociation Factor	Concentration			
		Percent	Volume Molecular		
			Decimal	Fraction	Osmotic
Magnesium 0.1 normal					
$Ca(NO_3)_2$	1.94	0.0533	0.00325	*M*/ 307.7	0.0063
KNO_3	1.90	0.0133	0.00132	*M*/ 758.4	0.0025
KH_2PO_4	1.86	0.0133	0.00098	*M*/1021.8	0.0018
$MgSO_4$	2.00	0.0013	0.00011	*M*/9031.0	0.0002
Na_2SO_4	1.91	0.0145	0.00102	*M*/ 979.8	0.0019
NaCl	2.00	0.0070	0.00119	*M*/ 835.1	0.0024
Total salts		0.1027	0.00787	*M*/ 127.1	0.0151
Calcium 0.1 normal					
$Ca(NO_3)_2$	2.00	0.0053	0.00032	*M*/3077.1	0.0006
KNO_3	1.90	0.0133	0.00132	*M*/ 758.4	0.0025
KH_2PO_4	1.86	0.0133	0.00098	*M*/1021.8	0.0018
$NaNO_3$	1.93	0.0316	0.00371	*M*/ 269.1	0.0072
$Mg(NO_3)_2$	1.99	0.0164	0.00113	*M*/ 904.9	0.0022
Na_2SO_4	1.97	0.0058	0.00041	*M*/2449.5	0.0008
Total salts		0.0857	0.00787	*M*/ 127.1	0.0151
Potassium 0.1 normal					
$Ca(NO_3)_2$	1.94	0.0533	0.00325	*M*/ 307.7	0.0063
KH_2PO_4	1.88	0.0031	0.00023	*M*/5293.6	0.0004
$NaNO_3$	1.97	0.0109	0.00128	*M*/ 779.9	0.0025
NaH_2PO_4	1.91	0.0089	0.00074	*M*/1349.4	0.0014
$MgSO_4$	1.87	0.0133	0.00111	*M*/ 903.1	0.0022
NaCl	2.00	0.0067	0.00114	*M*/ 872.5	0.0023
Total salts		0.0962	0.00775	*M*/ 129.0	0.0151
Phosphorus 0.1 normal					
$Ca(NO_3)_2$	1.94	0.0533	0.00325	*M*/ 307.7	0.0063
KNO_3	1.90	0.0133	0.00132	*M*/ 758.4	0.0025
KH_2PO_4	1.94	0.0013	0.00010	*M*/10218.0	0.0002
KCl	2.00	0.0067	0.00090	*M*/ 1112.8	0.0018
$MgSO_4$	1.87	0.0133	0.00111	*M*/ 903.1	0.0022
NaCl	2.00	0.0062	0.00104	*M*/ 942.9	0.0021
Total salts		0.0941	0.00772	*M*/ 129.5	0.0151
Nitrogen 0.1 normal					
$Ca(NO_3)_2$	2.00	0.0064	0.00039	*M*/2564.1	0.0008
KH_2PO_4	1.86	0.0133	0.00098	*M*/1021.8	0.0018
KCl	1.99	0.0098	0.00131	*M*/ 760.8	0.0026
$CaCl_2$	1.91	0.0399	0.00359	*M*/ 278.2	0.0068
$MgSO_4$	1.87	0.0133	0.00111	*M*/ 903.1	0.0022
Na_2SO_4	1.97	0.0065	0.00046	*M*/2185.7	0.0009
Total salts		0.0892	0.00784	*M*/ 127.6	0.0151

found practicable, on account of these difficulties, to take into account the effect of one constituent salt upon the dissociation of another in the culture solution, but the degree of dissociation in the various solutions was checked by measurements of electrical conductivity and also by freezing-point determinations.

Samples of the culture solutions for the various measurements were obtained at the termination of the year's experiments. The solutions were thoroughly shaken and filtered through ashless paper without previous wetting of the filter or washing of the residue. The first 500 cc. was discarded and a quantity was then obtained from which samples were taken for all the measurements.

The approximate total amount of soluble salts present in the solutions was determined by evaporating duplicate 10 cc. samples to dryness upon the water-bath. About 0.5 gram of zinc dust was added to each sample as a means of retaining as far as possible the nitric acid which might otherwise be lost on the water-bath and in subsequent drying. The residues were dried at 150° C., cooled in a desiccator, and weighed. The results, corrected for hydrogen evolved, are given in table 4.

The depression of the freezing-point of the different solutions was measured by means of a special Beckman apparatus, care being taken to avoid supercooling below 0.2° C. Readings were obtained that checked within 0.002° C. upon five samples from each solution (table 4).

Electrical conductivity measurements were made of all the solutions with a standard Kohlrausch apparatus. The average conductivity in reciprocal ohms is given in table 4.

TABLE 4

The Amounts of Salts in Solution, Electrical Conductivity, and Depression of the Freezing-Point of the Normal and Modified Nutrient Solutions.

Solution, Deficient Element Given	Total Soluble Salts in Grams per 100 Cc. of Solution			Conductivity × 10^{-6} Reciprocal Ohms			Depression of Freezing-point in Degrees Centigrade.		
	1916 Sol.	1917 Sol.	Ave.	1916 Sol.	1917 Sol.	Ave.	1916 Sol.	1917 Sol.	Ave.
Normal	.110	.105	.107	1.363	1.507	1.435	.050	.051	.050
Mg 0.1		.105			1.556			.051	
Ca 0.1	.085	.070	.077	1.144	1.058	1.101	.050	.050	.050
K 0.1	.109	.082	.095	1.343	1.451	1.397	.050	.051	.050
P 0.1	.098	.090	.094	1.383	1.305	1.344	.050	.051	.050
N 0.1	.120	.109	.114	1.455	1.625	1.540	.050	.061	.055

A comparison of the above tabulated results shows that the concentration of soluble salts is low in the solution in which calcium is deficient and comparatively high in the nitrogen-deficient solution. The lowering of the freezing-point caused by the respective solutions indicates a concentration in each case comparable to that expressed by the total soluble salts, but with less variation. The conductivity measurements, however, express variations in concentration notably similar to those expressed by the soluble salts (table 4 and fig. 1),

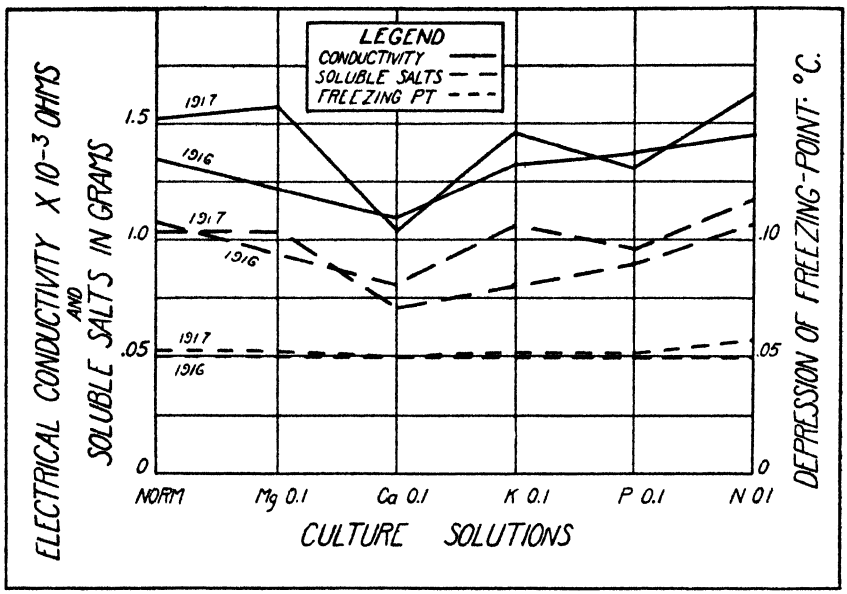


FIGURE 1. Curves showing the amount of salts in solution, electrical conductivity, and depression of the freezing-point in the normal and modified nutrient solutions.

which fact demonstrates that, at the dilute concentrations used in this work, the conductivity method supplies a very rapid and accurate method of determining the approximate concentration of a culture solution, since at these dilutions hydrolysis and other factors do not appear seriously to affect the conductivity.

GENERAL CULTURE METHODS

During the years 1915 and 1916 glazed earthenware jars of two-gallon capacity were used for the containers. During the year 1917

jars of four-gallon capacity were substituted for the smaller ones, allowing a greater production of plant substance since the number of plants was increased in proportion to the increase in size of the containers.

The plants were grown in quartz sand, 98 percent of which passed through a sieve of 0.5 mm. mesh. The sand was digested six hours in 10 percent hydrochloric acid and washed free of chlorides with distilled water. The composition of this washed sand was as follows:

SiO_2	99.98%
$FeCl_3$	.014
CaO	trace
K_2O	none
MgO	none
P_2O_5	none

The average moisture-holding capacity of the air-dried sand after the above-described treatment was 25.1 percent by weight.

Each jar was arranged with a four-inch glass funnel inverted in the bottom, into the stem of which was inserted a glass tube of one quarter inch diameter that extended above the top of the jar. A definite amount of sand was then poured around the funnel, after which the jars were covered with heavy paper until they were used for planting.

Pedigreed Swedish select oats, *Avena sativa aristata*, were used in all of the experiments. The seed was sterilized *in vacuo* with 0.25 percent mercuric chloride solution and washed in sterile distilled water until free from chlorides. The method of sterilization was similar to that employed by Hutchinson and Miller (1908), and when plated in bouillon agar after this treatment the seed showed no sign of infection. The seed after sterilization was germinated between cotton towels moistened with sterile distilled water until the primary root protruded beyond the seed coats. The seed was then again washed and planted; three plants were placed in each pot the first season, four the second, and eight the third. The sand in the jars, just previous to the planting of the seedlings, had been brought to 60 percent of its moisture-holding capacity by pouring the culture solution over the surface of the sand, the weight of the jars being recorded. After planting the seedlings, the jars were again covered and allowed to stand until the seedlings appeared above the surface of the sand, when the jars were put on the balance and brought to their former moisture content with distilled water. A small waxed-paper tube was placed

around each plant and a paraffin seal run over the top of the moist sand. The tubes were then plugged with cotton to prevent evaporation from the space not occupied by the stem, and the jars were placed in position under the open greenhouse.

The solution in the culture jars was maintained as nearly as possible at 60 percent of the moisture-holding capacity of the sand, the pots being brought to their former weight by pouring the culture solution through the glass tube into the inverted funnel. The jars were aërated by forcing air through this watering tube once a week.

The plants were grown under these conditions until completely matured, a period of about ninety days, from May until August, each year. Frequent observations were made and careful notes taken of the development until the plants were well ripened, when the final data were obtained. In all cases, the essential data are given in the following tables or in the text in connection therewith. The results given in the tables are averages of a series of checks. The number of determinations is given in each instance, with the mean variation following. Peters's abridged method (Briggs and Schantz, 1914), based upon the sum of the departures from the mean, was used to calculate the probable error of the mean as given in the formula

$$Rm = 0.845 \frac{\Sigma d}{n\sqrt{n-1}},$$

in which Rm = the probable error of the mean, Σd the sum of the departures, and n the number of determinations.

The Relation between Nutrients and Growth

The effect of limiting the essential mineral elements becomes apparent soon after the seedlings are transplanted. The plants grown in the solutions deficient in magnesium or in calcium stool heavily five days before those grown in the normal solutions. Those grown in potassium-deficient solutions, on the other hand, produce plants which stool slightly less heavily than the plants in the normal solutions. Each seedling in the solutions deficient in phosphorus or in nitrogen produces but one slender shoot.

The general appearance of the plants is markedly affected even before stooling begins, and during the subsequent development very

characteristic constant modifications are shown. The plants grown in the culture solutions deficient in magnesium develop numerous broad leaves, which are at first very bright green but when older show marked striping as the chlorophyll between the veins disappears.

The plants grown in the culture solutions deficient in calcium develop numerous dark green leaves nearly as long as those of the plants growing in the magnesium-deficient solutions. The lower leaves, however, soon show an inrolling of the margin, a characteristic which Loew (1899) and Tottingham (1914) ascribe to a high magnesium-calcium ratio. Later, at about the time of flowering, brown spots appear upon these inrolled leaves and finally the affected leaves dry and become twisted about the stem. Schimper (1890) describes brown spots on the leaves of Tradescantia grown in the absence of calcium, and von Portheim (1901) notes the appearance of similar spots on the leaves of beans grown in calcium-deficient soils.

The plants grown in the culture solutions deficient in potassium have a smaller total leaf surface than those grown in the normal solutions, but their leaves have very thick dark green laminae. During the period of blossoming, the lower leaves of these plants show long, irregular dark-brown blotches, which appear first near the leaf-base and gradually extend towards the apex.

The plants grown in the culture solutions deficient in phosphorus develop a slender culm which bears a few dwarfed, fleshy leaves. Both the leaves and stems are purplish green, with the purple color apparently gradually replacing the green in the older tissues of the plant. The abnormal coloring appears to be brought about by the presence in the reticulum of the chloroplast of a purple colloidal substance intermixed with the chlorophyll, and resulting perhaps from a progressive decomposition of the latter. Russell (1913) has shown that when barley is grown in phosphorus-free solutions the stems become reddish near the apex.

The plants grown in the solutions weak in nitrogen produce on an average four narrow, yellowish green leaves. Upon microscopic examination, the chloroplasts of these leaves appear to be more or less disorganized.

The period of development is shortened by fully a week when nitrogen or phosphorus is limited, but a deficiency in magnesium or in calcium increases the period of growth about ten days beyond that of the plants grown in the normal solutions.

In general, magnesium- or calcium-deficiency affects least the vegetative development of the plants; in some cases a deficiency in either of these elements causes even an increased growth. In potassium-deficient solutions the growth of the plants is less than that of the plants grown in normal solutions, although the effects are not marked in any particular instance. A deficiency in phosphorus or in nitrogen, however, produces a markedly unfavorable effect upon growth. The relation of the various elements to the general vegetative development of the plants is shown in figure 2.

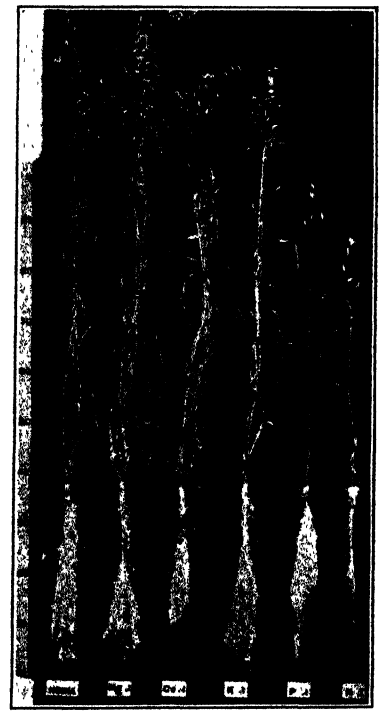


FIGURE 2. Photograph showing the growth of oats in the normal solution and in solutions with one essential element in each case reduced to one tenth normal.

THE RELATION BETWEEN NUTRIENTS AND TOTAL DRY WEIGHT

The final vegetative growth and the total dry weight of the plants in the respective cultures at maturity correspond quite closely with the growth of the plants after stooing (at the end of twenty days of growth). Certain specific differences, however, such as the amount of grain produced, the ratio of grain to straw, and the weight of the individual kernels under varying nutritive conditions, are brought out only by carrying the plants to maturity. The plants grown in solutions deficient in magnesium produce a greater total vegetative growth than those grown in normal solutions. The plants grown in solutions deficient in calcium show a total vegetative growth equal to that of plants grown in normal solutions. It is evident, therefore, that if a very small amount of magnesium and calcium is present in the culture solution (one tenth of that in the normal solution), the vegetative development of the plant will not be greatly affected, unless

the magnesium-calcium ratio is high enough to exert a toxic effect upon the plant. A deficiency in potassium, however, causes a decrease in the vegetative growth of the oat plant, and also affects the total dry weight which in this case is only about one half that of the plants grown in the normal solution. A deficiency in phosphorus or in nitrogen also causes a very great decrease in vegetative growth. There is no very marked difference in this respect between the effects of a deficiency in phosphorus and those of a deficiency in nitrogen.

TABLE 5

The Average Yields of Dry Weight by Plants Grown in the Normal Solution and in Solutions in Which Certain Essential Nutrients Are Reduced to One Tenth the Normal Amount

No. Det. per Year	Solution, Deficient Element Given	Weight of Dry Matter in Grams			
		1915 Crop	1916 Crop	1917 Crop	Ave.
4	Normal	12.41 ± 1	20.90 ± 2	34.44 ± 0	22.58
2	Mg 0.1			35.42 ± 3	
2	Ca 0.1	13.17 ± 1	23.85 ± 4	24.74 ± 3	20.59
2	K 0.1	6.48 ± 3	15.88 ± 6	15.99 ± 0	12.77
2	P 0.1	2.73 ± 1	1.23 ± 1	2.77 ± 0	2.24
2	N 0.1	4.00 ± 1	.41 ± 0	2.70 ± 0	2.37

The comparative effect of a deficiency in the respective nutrient elements upon the total dry weight of dry matter is presented graphically in figure 3.

THE RELATION BETWEEN NUTRIENTS AND GRAIN PRODUCTION

Although the total dry weight of the plants grown in the solutions deficient in magnesium or in calcium is the same or even greater than that of the plants grown in the normal solution, yet the total dry weight of the grain produced by the plants grown in the former solutions is lower than that produced by plants grown in the normal solution (table 6). The ratio of grain to straw for the plants grown in magnesium- or in calcium-deficient solutions is lower than that of plants grown in the normal solutions. The weight of the individual kernels of the plants grown in the magnesium- or in the calcium-deficient solutions is also below that of the kernels of plants grown in the normal solution (table 7). A deficiency in magnesium or in calcium, therefore, has a markedly deleterious effect upon the grain production of the oat plant.

It is held by some that magnesium or calcium deficiency causes a disturbance in the translocation of carbohydrates and proteins and in their storage during seed formation, rather than an interruption in their synthesis, providing sufficient magnesium is present for the formation of chlorophyll. The results of the experiments reported

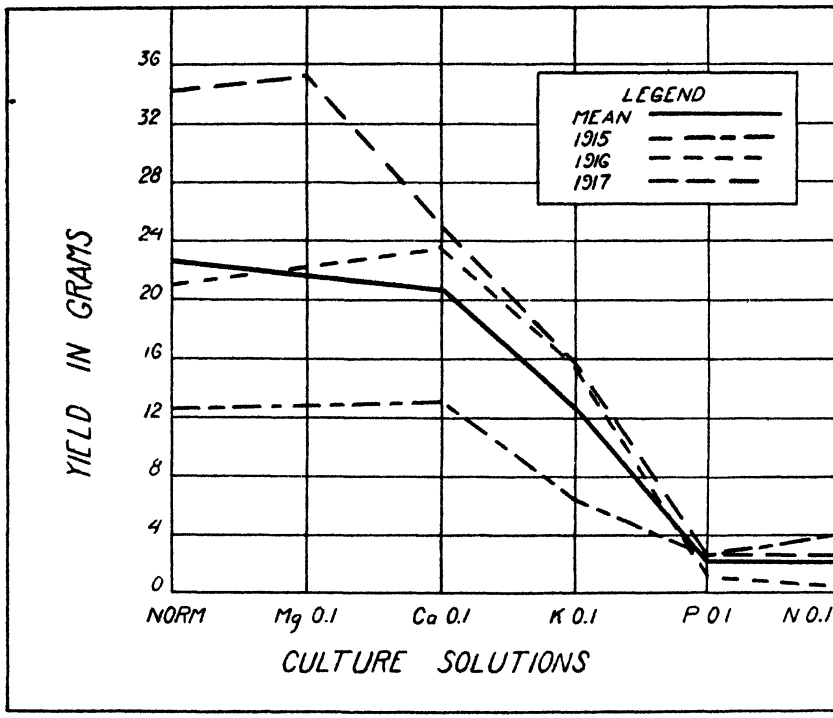


FIGURE 3. Curves showing the average yields of dry matter by plants grown in the normal solution and in solutions in which one nutrient element in each case is reduced to one tenth the normal amount.

in the present paper quite substantially support this theory, since the plants in the magnesium- or in the calcium-deficient solutions grow vigorously until the period of seed formation. At the beginning of this phase of their development, however, a deficiency in magnesium or in calcium causes a deleterious effect and results in low grain production.

A further proof that magnesium functions in some manner in

food translocation and storage is that analyses show an abundance of magnesium in the seeds of plants. It has been shown that magnesium occurs chemically combined with many of the fats and some of the phospho-nitrogen compounds. It is possible, therefore, that the major rôle of magnesium in plant nutrition is in connection with seed formation, and if this be true, it is evident that a deficiency in this element in the culture solution will affect seed production rather than vegetative growth. On the other hand, although calcium apparently plays no very important part in the chemical composition of the seed, yet when this element is deficient in the culture solution a marked decrease results in the amount of grain produced. The decrease in the amount of grain produced in calcium-deficient solutions is nearly as great as that noted in magnesium-deficient solutions. It would appear, therefore, that calcium does not function directly in the synthesis of carbohydrates and proteins, but rather as a neutralizer of acids in the plant and as a carrier for nitrogen, phosphorus, and sulphur compounds, a view first advanced by Holzner (1867) and Schimper (1890), and more recently supported by Chirikov (1914) and Robert (1917).

Truog (1916) finds that plants with a high protein content generally have a high calcium content, and that when manganese phosphate is used instead of calcium phosphate as a source of phosphorus the plants grown in such a solution have an extraordinarily high manganese content. Although neither calcium nor manganese is an important constituent of the proteins of the protoplasm, and although neither enters into direct chemical combination with any of the more important compounds of the seed, yet their presence in plants especially high in nitrogen content must indicate some connection with protein formation. In the synthesis of proteins organic acids are formed which must be neutralized by some base to form relatively insoluble salts; calcium, therefore, may be imagined to be the base which chiefly functions in removing these acids from the field of action. From this point of view it would appear that if the nutrient solution is deficient in calcium, and if no other base is present which will form insoluble salts with the organic acids in sufficient quantity to remove the latter, then the normal development of the plant will be disturbed by the accumulation of the acids in sufficient quantities to exert a retarding effect upon the plant's metabolism.

The plants grown in the solutions deficient in potassium produce only about one half the amount of total dry matter produced by the

plants grown in the normal solutions. However, the dry weight of the grain produced by the former plants is two thirds of the dry weight of the grain produced by those grown in the normal solutions. The decrease in dry weight of grain resulting from the growing of plants in potassium-deficient solutions is therefore not in proportion to the decrease in the production of total dry matter; for although fewer kernels are formed when plants are grown in potassium-deficient solutions, those that are formed are heavier than an equal number of kernels from plants grown in the normal solutions. Grain formation takes place at the expense of vegetative growth.

The high grain-straw ratio in plants grown in potassium-deficient solutions may be partially explained by tracing the movement of potassium during the development of the plant. Analyses show that potassium is localized first in the growing regions of the young seedling; later, during the development of the flower, it appears in large quantities in the region of grain formation; and finally it is stored in the mature kernel rather than in the straw. As the potassium salts are very soluble, they are readily transferred from the embryonic tissues of leaves and stems to the embryonic region of the flower. Since an abundance of potassium seems to be necessary for meristematic development, the activity of the embryonic regions of the vegetative parts will be checked by the decrease in their potassium supply during flower formation, the result being a high grain-straw ratio.

TABLE 6

The Average Yield of Grain and Straw in Plants Grown in the Normal Solution and in Solutions with One Nutrient Element in Each Case Reduced to One Tenth Normal.

No. Det. per Year	Solution, Deficient Element Given	Weight of Grain in Gm.			Weight of Straw in Gm.		
		1915 Crop	1916 Crop	1917 Crop	1915 Crop	1916 Crop	1917 Crop
4	Normal	5.329 ± 2	6.986 ± 1	6.362 ± 0	7.08 ± 2	14.77 ± 2	28.08 ± 0
2	Mg 0.1			5.804 ± 1			29.62 ± 2
2	Ca 0.1	5.212 ± 1	6.645 ± 0	3.999 ± 0	7.96 ± 2	17.21 ± 3	20.75 ± 2
2	K 0.1	2.968 ± 1	5.660 ± 2	3.268 ± 0	3.51 ± 2	10.22 ± 4	12.68 ± 0
2	P 0.1	1.142 ± 1	.390 ± 1	.665 ± 0	1.59 ± 1	.84 ± 1	2.11 ± 2
2	N 0.1	1.767 ± 1	.120 ± 1	.679 ± 0	2.23 ± 1	.29 ± 0	2.02 ± 0

The general decrease in production of dry matter by plants grown in potassium-deficient solutions may be due to the fact that potassium acts in some way as a condenser or catalyzer in the process of translocation and subsequent condensation of the carbohydrates and

proteins. Since phosphorus and nitrogen enter into the chemical composition of compounds found in both foliage and fruit, a deficiency in either of these elements in the culture solution limits the total weight of the grain produced as well as the total dry weight of the plant.

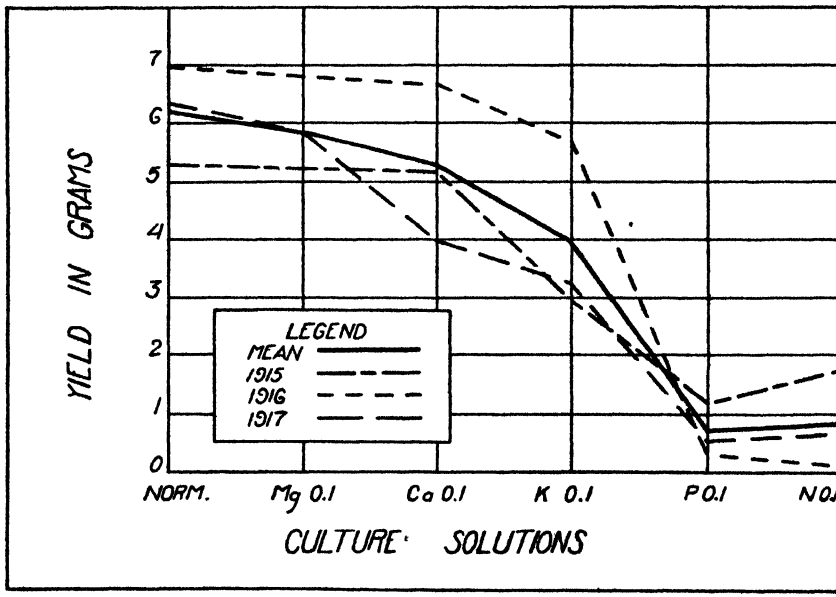


FIGURE 4. Curves showing the average yield of grain by plants grown in the normal solution and in solutions with one nutrient element in each case reduced to one tenth normal.

TABLE 7

The Average Ratio of Grain to Straw and the Weight of the Individual Kernels Produced by Plants Grown in Normal Solution and in Solutions with One Nutrient Element in Each Case Reduced to One Tenth Normal

No. Det. per Year.	Solution, Deficient Element Given	Grain/Straw Ratio (in Percentage)				Weights of Individual Kernels in Grams.			
		1915	1916	1917	Ave.	1915	1916	1917	Ave.
4	Normal	75.3	47.3	22.7	48.4	.0204	.0181	.0092	.0159
2	Mg 0.1			19.6				.0081	
2	Ca 0.1	65.5	38.6	19.3	41.1	.0198	.0143	.0089	.0143
2	K 0.1	84.5	55.4	25.8	55.2	.0245	.0175	.0115	.0178
2	P 0.1	71.9	46.4	31.5	50.0	.0238	.0230	.0101	.0190
2	N 0.1	79.3	41.4	33.1	51.3	.0253	.0200	.0186	.0213

Although the ratio of dry weight of grain to dry weight of straw during the three consecutive years varies widely as shown in table 7, yet the effect upon this ratio of a deficiency in any particular nutritive element of the culture solution is similar throughout the three years. The tendency of plants to fruit normally even when their vegetative development is very greatly disturbed is brought out strikingly in the results presented in table 7. Plants grown in magnesium- or in calcium-deficient solutions, provided their vegetative growth is not abnormal, produce an abundance of straw and a relatively small amount of grain. As shown by the weights of the individual kernels in the last four columns of table 7, the grain produced in such solutions is very light. Plants grown in solutions deficient in potassium, phosphorus, or nitrogen, on the other hand, produce a higher ratio of grain to straw, and their kernels are very much heavier than those of plants grown in normal solutions.

Hellriegel and Wilfarth (1888) report that the production of total dry matter and grain are greatly decreased when either potassium, phosphorus, or nitrogen is deficient. Their data show further that the ratio of grain to straw and the weights of the individual kernels of plants grown in solutions deficient in phosphorus or nitrogen are higher than those of plants grown in normal solutions. They record, however, a marked decrease in the ratio of grain to straw and in the weight of the individual kernels in plants grown in solutions deficient in potassium. My own results are at variance with those of some of these experiments, as is shown by the effects of a deficiency in potassium upon the ratio of grain to straw and upon the weight of individual kernels. The data presented in table 7 show that plants grown in solutions deficient in potassium have the highest ratio of grain to straw and that they bear kernels considerably heavier than those of plants grown in normal solutions.

The seasonal variation in the ratio of grain to straw may be partially explained by climatic differences under which the plants were grown for the three seasons. These variations will be discussed in a later paper.

The Relation between Nutrients and Water Requirement

It has long been known that there exists normally a fairly definite ratio between transpiration and growth, and that total transpiration

under constant conditions is a more or less accurate criterion of total growth. Many external as well as internal factors, however, influence the rate and amount of transpiration, making the water requirement of any plant very variable. Any particular crop will show a varying water requirement dependent upon temperature, light intensity, concentration of soil solution, and other factors. In a study of the relation of nutrient elements to water requirements, therefore, the necessity is evident of having all other conditions as nearly constant as possible. Great care has been taken in the work here reported to eliminate all environmental variations except the concentration of the mineral nutrients.

In general, a decrease in the normal concentration of the soil solution or in that of any essential nutrient element therein causes an increase in the water requirement of the plants growing upon the soil in question. Hellriegel (1883) finds that the water requirement for oats is doubled by the absence of potassium and trebled by a deficiency in nitrogen. He adds that in general an abnormally high water requirement is to be expected of a plant growing in a soil deficient in any essential element, because growth would be arrested while transpiration would still continue. The experiments herein described and many others of a similar nature show that in general the water requirement of crops is in inverse ratio to the amount of the limiting element in the culture solution.

TABLE 8

The Average Water Requirement, Based Upon the Dry Matter Produced, of Plants Grown in the Normal Solution and in Solutions with One Nutrient Element in Each Case Reduced to One Tenth Normal

No. Det. per Yr.	Solution, Deficient Element Given	Cubic Centimeters of Water Required to Produce a Gram of Dry Matter			
		1915 Crop	1916 Crop	1917 Crop	Average
4	Normal	516 ± 12	440 ± 8	401 ± 2	452
2	Mg 0.1			400 ± 2	
2	Ca 0.1	502 ± 2	407 ± 2	494 ± 1	468
2	K 0.1	614 ± 7	471 ± 2	478 ± 1	521
2	P 0.1	717 ± 5	741 ± 2	832 ± 8	763
2	N 0.1	839 ± 15	1330 ± 14	644 ± 8	938

In this series of experiments, the water requirement, based upon the dry weight of plants at maturity, is enhanced by a decrease in any one of the mineral nutrients with the exception of magnesium (table 8).

The water requirement of the plants grown in the magnesium-deficient solutions is 52 cc. less than the average water requirement of the plants grown in the normal solutions, but the data of one year, 1917, show no perceptible difference between the water requirement of the plants grown in the magnesium-deficient solutions and those in the normal solution. Calcium-deficiency causes a slight increase in the water

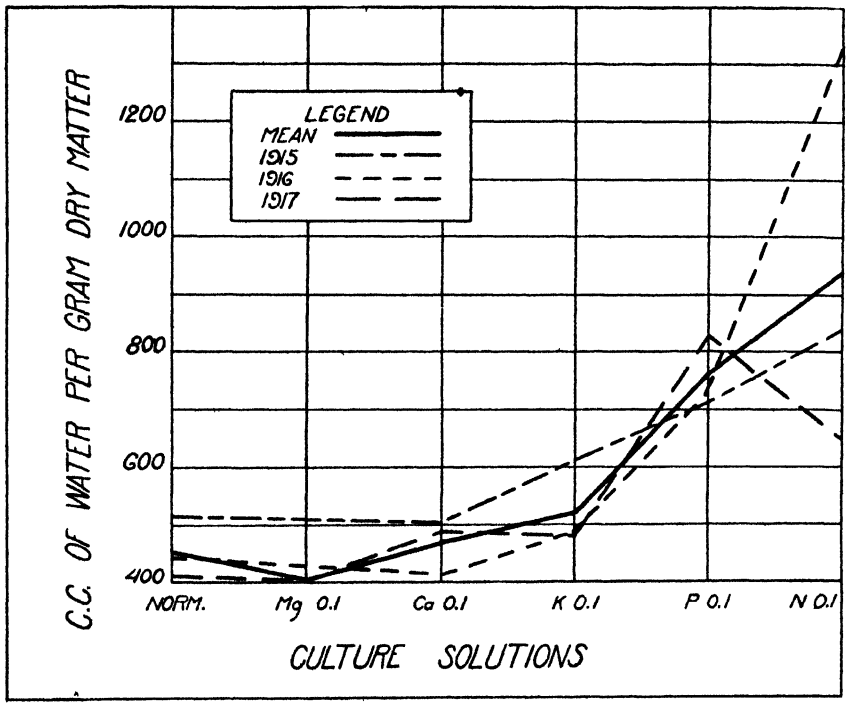


FIGURE 5. Curve showing the average water requirement of plants grown in the normal solution and in solutions with one nutrient element in each case reduced to one tenth normal.

requirement of the plants when an average of three years' experiments is considered, but in the experiments of the first two years the water requirement of plants grown in the calcium-deficient solutions is less than that of those grown in the normal solution. The average water requirement of plants grown in potassium-deficient solutions is 69 cc. above the water requirement of the plants grown in the normal solution. The water requirement of plants grown in phosphorus-deficient

solutions is nearly doubled, and for those grown in nitrogen-deficient solutions it is more than trebled. Figure 5 shows the general relation of the respective nutrient elements to the economic use of water by the oat plant.

SUMMARY

1. The general development of the plants studied is most severely affected by a deficiency in phosphorus or nitrogen.

2. A deficiency in phosphorus or nitrogen prevents the stooling of the plants.

3. The general vigor of growth is increased by a deficiency in magnesium or calcium, and is greatly decreased by a deficiency in phosphorus or nitrogen.

4. The period of development is shortened by a deficiency in potassium, phosphorus, or nitrogen, and is lengthened by a deficiency in magnesium or calcium.

5. The total dry weight of the plants is greater than the normal when magnesium or calcium is deficient, and less than the normal when potassium, phosphorus, or nitrogen is deficient.

6. Grain production is lowered by a deficiency in any one of the elements in question: magnesium, calcium, potassium, phosphorus, and nitrogen.

7. The ratio of grain to straw is decreased by a deficiency in magnesium or calcium, and is increased by a deficiency in potassium, phosphorus, or nitrogen.

8. The weight of the individual kernels is lowered by a deficiency in magnesium or calcium, and is raised by a deficiency in potassium, phosphorus, or nitrogen.

9. The water requirement of the plants is decreased by a deficiency in magnesium, slightly increased by a deficiency in calcium, and greatly increased by a deficiency in potassium, phosphorus, or nitrogen.

10. In general, the effects upon the plants of limiting the supply of phosphorus or nitrogen are much more noticeable than the effects of limiting the supply of magnesium, calcium, or potassium.

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UREDINALES OF GUATEMALA BASED ON COLLECTIONS BY E. W. D. HOLWAY

I. Introduction, Coleosporiaceae and Uredinaceae

J. C. Arthur

Guatemala, the largest of the Central American republics, and with much more than one third of their total population, is a land of great charm for the traveler and the naturalist. The hot, low lands near the coast, especially on the Atlantic side, with their dense tropical growth, the extensive plateau of the interior, ranging from 8,000 feet elevation in the north to half that altitude in the south, thus supplying a temperate climate, and the many high mountains with their pine-clad summits and cool breezes, give a wonderful range for all forms of vegetation. The large proportion of Indians among the population, the many cities of twenty-five to seventy-five thousand inhabitants, the diversity of landscape, and the enjoyable climate lend a special fascination to the task of the explorer.

The rusts of Guatemala have been made known through the efforts of two tireless collectors of superior botanical attainments, who gave the Uredinales their first attention, endeavoring to take ample specimens to illustrate both the rust and its host, but who gathered also other fungi, as well as higher forms, especially phanerogams. Not a dozen collections of Guatemalan rusts are known from all other sources taken together.

Professor W. A. Kellerman made four visits to Guatemala at the beginning of the years 1905, 1906, 1907 and 1908, and was so enamored of the country and its interesting vegetation that upon returning from his third trip in April, 1907, he laid plans for a peripatetic school of tropical botany. On his next visit he took with him a few students from the University of Ohio. The program for this visit had been completed and arrangements made for departure homeward when a brief illness terminated the career of a zealous and undaunted collector. The larger part of the rich material secured during these four years yet remains unstudied. Two papers dealing with the rust por-

tion have been published by Dr. Frank D. Kern,[1] the first one enumerating forty species, five being described as new, and the second giving fifteen species, one being described as new. Two decades of fungi were issued by Professor Kellerman,[2] together containing seventeen species of rusts. All the collections listed in these four publications and a few others are cited in the following pages, making a total of 112 of the Kellerman mycological numbers.

Professor E. W. D. Holway has made three visits to Guatemala: December 30, 1914, to February 10, 1915; February 7 to March 23, 1916; and December 19, 1916, to February 18, 1917. The last visit, although the longest of the three, was brought to a premature termination by the disturbing influences of the European war. A total of 600 rust numbers has resulted from these explorations, and a duplicate set of this rich lot of material was placed without restrictions in the hands of the writer for study. As will be seen by the following enumeration there are considerably more than two hundred species represented, that is, every third specimen collected supplied an additional species. In order to make a full showing of all Guatemalan rusts known up to the present time, there have been added eighteen species from the Kellerman material not taken by Professor Holway, and four such species from other sources.

Considering the area of country involved and the comparatively limited amount of exploration, the list of species here presented doubtless indicates a richer rust flora for Guatemala than for any similar area on the North American continent, although it is the opinion of Professor Holway, based on field experiences, that southern Mexico is the real paradise for the collector of rusts. No comprehensive account of Mexican rusts has yet been published, and statistical comparison is not at present possible.

Professor Holway entered Guatemala each time at the Atlantic port of Barrios, and made good use of the country's five hundred miles of railway. He also explored by other means of transportation and particularly by the aid of mules and Indian guides. Professor Holway possesses in a high degree those accessory qualifications of a successful collector, unbounded enthusiasm, keen enjoyment of the beautiful,

[1] Kern, F. D. The rusts of Guatemala. Journ. Myc. 13: 18–26. 1907; The rusts of Guatemala II. Mycologia 3: 288–290. 1911.

[2] Kellerman, W. A. Fungi selecti Guatemalensis exsiccati. Journ. Myc. 12: 238–241. 1906; 13: 99. 1907.

and a buoyant disposition that makes light of hardships. A few brief excerpts from his letters to the author will illustrate the manner in which he searched for rusts with such eminent success.

A week after reaching Guatemala City on his first visit, Professor Holway started for Antigua "over a road with two-foot holes and two-foot boulders and much dust," as he says, and at a lunch station, "within ten rods of the hotel, collected twenty-five rusts." The next day he writes from San Rafael: "Arrived here about noon, and although the afternoon was misty and dark I found about fifteen more rusts." Two days later he writes from Antigua: "Grand place! Volcanoes 8,000 feet directly above the town. Out three hours and found everything rusted here that was *not* at San Rafael." A single extract may be taken from the letters of the second trip. On February 22 he writes from Mazatenango. "This is the most surprising place! A fine, perfectly clean hotel, good food, no mosquitoes, the grandest and most luxuriant vegetation, and fine views of the Volcans de Atitlan and Santa Maria! There *is* a fly in the ointment—ticks the botanist has always with him." On his third trip, a letter written the middle of January says: "I wish you could have been with me at San Felipe and seen the Volcan de Santa Maria, the fine tree ferns, the brilliant orchids blooming on the tree trunks, etc., etc.—There *were* some ticks." Upon reaching Huehuetenango, a much more northern locality than any hitherto explored, ninety miles from the railroad, that is "three days' mule ride," and which promised to be especially rich in rusts, a telephone message was received giving warning of the changed attitude toward foreigners due to recent developments in the war. It was deemed advisable to make a hasty departure for Guatemala City, and as soon as arrangements could be made a steamer was taken from the west coast for return to the United States.

In the last few years there have been a number of notable explorations for rusts in the different parts of the American tropics, but doubtless none of them has yielded so rich a harvest of additional species for the North American flora as the work of Professor Holway in Guatemala. Part of these new species are forms previously known only from South America, but very many more are species new to science. As a presentation of the rust flora of Guatemala, however, the list as it now stands must be accepted as only a good beginning. Even the species given in many cases require the discovery of additional stages in order to make known their full life cycle. It must also

be remembered that much of the northern part of Guatemala yet remains to be explored, especially the great department of Petén which includes nearly one third of the area of the country and is botanically a veritable *terra incognita*. Even the better known parts will yield many more species, especially the cool summits of the high mountains. As Professor Holway wrote in May, 1917, "the Volcan de Santa Maria is very rich and has only been scratched;" and the same might be said of other localities in this enumeration, even those most frequently mentioned.

The author has been assisted in the study of the Guatemalan material not only by Professor Holway, but also by various members of the botanical staff of the Purdue University Agricultural Experiment Station, working in connection with the preparation of the rust portion of the North American Flora, to whom many thanks are due, and especially to Professor H. S. Jackson and Dr. E. B. Mains, who have described some of the species.

In order to bring out more clearly the several groups of rusts, their relationships, and the association of the new species, the list of species will be presented in several parts. The first part includes twenty-two species belonging to the families Coleosporiaceae and Uredinaceae. This group is more notable for its familiar names than for novelties. The heteroecious species, *Coleosporium Ipomoeae*, *C. Viburni*, *Melampsora Bigelowii*, *M. arctica*, *Cronartium Quercus* and *C. coleosporioides*, common in the northern United States, seem to be abundant in this tropical country. It should be noted, however, that with the exception of the last-named only uredinia are recorded. Pines are common enough in the region, especially at higher altitudes, but little search has yet been made for aecia (Peridermiums) on them at the season of the year when they are most likely to be found. The aecia possibly may be rare or wanting for these species so far south, unless *Cronartium coleosporioides*, which shows telia, is an exception. New hosts are recorded for this last species.

Finding the common grape-vine rust of warmer regions on a native species may prove to be a matter of economic importance. Although first reported from America, the principal observations regarding the nature of the rust have been made in India and Japan.

Family: **Coleosporiaceae**

Coleosporium domingensis (Berk.) comb. nov. (on Apocynaceae).

Plumiera lutea Ruiz & Pav.

Plumiera rubra L.

A specimen of this rust on *P. lutea* is in the cryptogamic herbarium of the New York Botanical Garden, collected by J. Donnell Smith, at Cuyatenango, April, 1892, showing uredinia. A collection on *P. rubra* was made by Kellerman, at Palmar, Dept. Quezaltenango, Feb. 11, 1906, II, *5460*, reported by Kern, Journ. Myc. **13**: 18-26. 1907, and issued in Kellerman's Fungi Selecti Guatemalensis *13*, under the name *C. Plumierae* Pat.

The species is apparently abundant in the West Indies, but these two collections are the only ones known from the continent. Telia are seemingly rare. The aecia doubtless appear on pine leaves, whenever formed, as in all other species belonging to the genus Coleosporium, but no trace of them has yet been secured.

The director of the Royal Kew Herbarium kindly sent the writer recently a fragment from the type material of *Uredo domingensis* Berk., published in 1852, in the Ann. Mag. Nat. Hist., 2d series, vol. 9, as on an unknown plant from the West Indian island of Santo Domingo. Although the fragment sent was only a centimeter square, it bore numerous sori in good condition. It was easy to see that the fungus was the uredinial stage of a Coleosporium. From the peculiar areolation of the smooth surfaces of the leaf it was possible tentatively to refer the host to Plumiera. With the assistance of Mr. Percy Wilson, of the New York Botanical Garden, this assumption was confirmed, and it was further made highly probable that it belonged to *P. rubra*, but that could not be positively confirmed. The specific name of the rust is here changed to agree with this discovery. It has also been found that *Uredo plumieriicola* P. Henn. (Hedwigia **43** : 161. 1904) is to be referred to the same species.

2. Coleosporium Ipomoeae (Schw.) Burr. (on Convolvulaceae).

Ipomoea glabriuscula House, Sanarate, Dept. Guatemala, Feb. 10, 1916, II, III, *471*.

Ipomoea muricata Roem. & Schult., Guatemala City, Dec. 31, 1914, II, *1*.

Ipomoea Petri Donn. Sm. (*I. sericophylla* Peter, not Meissn.), San Lucas Toliman, 5,100 feet alt., Dept. Solola, Feb. 2, 1915, II, *181;* Moran, Dept. Amatitlan, Dec. 22, 1916, II, *619*.

It was collected by Kellerman, on *Ipomoea macrocalyx* (Ruiz & Pav.) Choisy, at Laguna, Dept. Amatitlan, Jan. 19, 1906, II, *5450*, and Jan. 20, 1906, II, *5408*, on *I. tyrianthina* Lindl., at Moran, Dept. Amatitlan, Jan. 25, 1906, II, *5435*, and on *Pharbitis hederacea* (L.) Roth, at Laguna, Jan. 17, 1906, II, *5405*, *5409*, and all reported by Kern in Journ. Myc., *l. c.*

It is a common rust in both tropical and temperate America. The connection of the aecial form on pine leaves has been proven by cultures.

3. COLEOSPORIUM VIBURNI Arth. (on Caprifoliaceae).
Viburnum sp., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, *567*, *574*.

A species of wide distribution in North and South America, but local and rarely collected. It undoubtedly has its aecia on leaves of pine, but they have not yet been detected.

4. COLEOSPORIUM ELEPHANTOPODIS (Schw.) Thüm. (on Carduaceae).
Elephantopus hypomalacus Blake, San Felipe, Dept. Retalhuleu, Jan. 13, 1917, II, III, *704*.

This species occurs in tropical North and South America. It was collected by Kellerman on *E. mollis*, at Los Amates, Dept. Izabal, March 15, 1905, II, *5362*, and reported by Kern in Journ. Myc., *l. c.*

5. COLEOSPORIUM EUPATORII Arth. (on Carduaceae).
Eupatorium collinum DC., Huehuetenango, Jan. 21, 1917, II, *758*.
Eupatorium sp., Tecpan, Dept. Chimaltenango, Jan. 1, 1917, II, *659*; Quezaltenango, Jan. 31, 1917, II, *812*.

Common on various species of Eupatorium in tropical North and South America. It was collected by Kellerman on *E. collinum*, at Palmar, Dept. Quezaltenango, Feb. 11, 1906, II, *5458*, and reported by Kern in Journ. Myc., *l. c.*

6. COLEOSPORIUM STEVIAE Arth. (on Carduaceae).
Stevia lucida Lag., Huehuetenango, Jan. 23, 1917, II, *772*.
Stevia subpubescens Lag., Cerro Quemado, Dept. Quezaltenango, Jan. 21, 1915, II, *104*.

This heteroecious species has not been reported before outside of Mexico. Aecia are not known for it.

7. COLEOSPORIUM VERBESINAE Diet. & Holw. (on Carduaceae).
Verbesina apleura Blake, Quezaltenango, Jan. 17, 1917, II, *739*.

Verbesina Holwayi Rob., Quezaltenango, Jan. 17, 1917, III, *737* (with *Puccinia cognata*).

Verbesina perymenioides Sch. Bip., San Lucas Toliman, 5,100 feet alt., Dept. Solola, Feb. 2, 1915, II, iii, *172*.

Verbesina scabriuscula Blake, San Felipe, Dept. Retalhuleu, Jan. 14, 1917, II, *723*.

Verbesina sublobata Benth., San Rafael, Dept. Guatemala, Jan. 9, 1915, II, *51;* San Lucas Toliman, 5,100 feet alt., Dept. Solola, Feb. 2, 1915, II, *175B* (with *Puccinia cognata*).

Verbesina sp., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, *575*.

The species was united with *C. Helianthi* in the N. Amer. Flora (7 : 89. 1907), but the cultures by Hedgcock and Hunt[3] indicate that it may be distinct, an assumption which is strengthened by the much more southern range, being common in Mexico, Central America and the West Indies, while the Coleosporium on Helianthus is not reported south of the United States. It was collected by Kellerman on *V. gigantea* Jacq., Patalul, Dept. Solola, Feb. 13, 1906, II, *5385*, and on *V. turbacensis* H. B. K., Los Amates, Dept. Izabal, March 15, 1905, II, *5315*, and reported by Kern in Journ. Myc., *l. c.*

8. Coleosporium paraphysatum Diet. & Holw. (on Carduaceae).

Liabum hypochlorum Blake, San Felipe, Dept. Retalhuleu, Jan. 13, 1917, II, III, *703*.

Liabum sublobatum Rob., San Lucas Toliman, Dept. Solola, Feb. 2, 1915, ii, III, *170;* Retalhuleu, Feb. 26, 1916, II, *532*.

Liabum sp., San Felipe, Dept. Retalhuleu, Jan. 12, 1917, II, *690*.

This heteroecious species has not been reported before outside of Mexico. Its aecia are unknown.

Family: **Uredinaceae (Melampsoraceae)**

9. Melampsora Bigelowii Thüm. (on Salicaceae).

Salix Bonplandiana Kunth, Quezaltenango, Jan. 18, 1917, II, *752*.

Salix Humboldtiana stipulacea Schn., Antigua, 5,300 feet alt., Dept. Sacatépequez, Jan. 11, 1915, II, *72*.

A heteroecious species with aecia on Larix, and very common in North America, especially in the uredinial stage.

The rust appears also to be common in Guatemala. It was col-

[3] An alternate form for Coleosporium Helianthi. Phytopath. 7: 67. 1917.

lected by Kellerman on *Salix Humboldtiana* H. B. K., near Patalul, Dept. Solola, Feb. 16, 1906, II, *5473* (Kellerm. Fungi Sel. Guat. *2*), and reported by Kern in Journ. Myc., *l. c.* The willow, *S. Humboldtiana*, forms conspicuous groves in the middle altitudes.

10. MELAMPSORA ARCTICA Rostr. (on Salicaceae).

Salix taxifolia microphylla Schn., Huehuetenango, Jan. 22, 1917, II, *763*.

This species of willow rust has small urediniospores, and is a somewhat common form in northern regions, especially in the mountains. It has aecia on Abies.

11. PHAKOPSORA VITIS (Thüm.) Syd. (on Vitaceae).

Vitis caribaea DC., Guatemala City, Jan. 7, 1917, II, *680*.

This is the first record of the rust occurring upon native grapes in America. The spores from this specimen in general are smaller than usual, and the paraphyses are noticeably thickened on the convex side. The latter character is not mentioned in the diagnosis given in the North American Flora (7 : 102), where it is listed under the name *Physopella Vitis*. It is a common tropical rust, whose life history is not fully known.

12. SPIRECHINA RUBI (Diet. & Holw.) Arth. (on Rosaceae).

Rubus laxus Rydb., Huehuetenango, Jan. 21, 1917, II_2, III, *756*.

Rubus Pringlei Rydb., Volcan de Agua, 7,000 feet alt., Dept. Sacatépequez, Jan. 13, 1915, II_2, *80;* same March 7, 1916, II_2, *559, 560*.

Rubus sp., San Rafael, Dept. Guatemala, Jan. 7, 1915, II_2, *17, 33;* Quezaltenango, Jan. 13, 1917, 0, II_1, II_2, III, *746*.

Not until the fine specimen of this rust on *Rubus laxus* was examined did the real differences between primary and secondary uredinia in this species manifest themselves. In this specimen the under surface of the leaf is evenly covered with orange telia, the color coming from the spore contents, the walls being colorless, while the upper surface is well covered with secondary uredinia, of course unaccompanied with pycnia. All the other collections except one here listed proved to have secondary uredinia only. Comparing these collections with others from Mexico, it was not difficult to make out that in this species both primary uredinia (with pycnia) and secondary uredinia (without pycnia) are epiphyllous, and do not noticeably differ morphologically either in sori or spores. The spores of both stages origi-

nate within the epidermal cells, which they stimulate into abnormal growth. When the overarching part of the host is pushed back from the sorus, only the outer wall or upper half of the epidermal layer is involved. The species, on account of its one-celled teliospores, is often listed as *Uromyces Rubi* Diet. & Holw.

The genus Spirechina is here placed in the Uredinaceae (Melampsoraceae) in association with Kuehneola. It has usually been placed near Phragmidium, because of its host affinities and the subcuticular pycnia, but both of these characters ally it equally well to Pucciniastrum. The occasional formation of intracellular urediniospores and teliospores also shows relationship to that genus. The lack of a uredinial peridium, however, places it, together with Kuehneola, in the subfamily Uredinatae, rather than in the Pucciniastratae.

The species was collected by Kellerman, on *Rubus glaucus* Benth., Guatemala City, Feb. 12, 1905, II, *4625;* between Antigua and Volcan de Agua, Feb. 15, 1905, II, III, *5321;* same, Feb. 18, 1905, II, iii, *5319,* and II, *5320;* on *R. poliophyllus* Focke, Volcan de Atitlan, Dept. Solola, Feb. 16, 1906, II, III, *5415;* same between Antigua and Volcan de Agua, Feb. 18, 1905, II, *5363,* all being reported by Kern in Mycologia. Numbers *4625* and *5363* were first erroneously reported by Kern in Journ. Myc., *l. c.,* as *Kuehneola albida,* a species not yet known south of the United States.

13. SPIRECHINA ARTHURI (Syd.) Arth. (on Rosaceae).

Rubus guyanensis Focke (?), Road between Quezaltenango and Colomba, Feb. 4, 1917, 0, II_1, II_2, iii, *832.*

This collection gives the first opportunity to study pycnia and uredinia of the species. Heretofore only a few urediniospores have been seen in connection with teliospores, independent uredinia not having been found.

The pycnia are amphigenous, scattered on gall-like swellings of the leaf, 0.2–1.5 cm. in diameter. They are subcuticular, golden- to chestnut-brown, discoid, 190–480μ broad by 48–80μ high; pycniospores ellipsoid, colorless, 3-4μ long.

The primary uredinia are on the pycnial galls, at first circinating, afterward becoming crowded, confluent, and covering the gall. The urediniospores are 16–23 by 30–42μ, with the walls colorless, about 1μ thick, thickened at the apex 7–18μ, and the apex rounded or acute.

The secondary uredinia are not on galls, but are scattered over the under surface of the leaf, round, 0.1–0.2 mm. across, pulverulent, yellow fading to white, with the ruptured epidermis inconspicuous.

The urediniospores are smaller than in the primary form, 16–19 by 24–35μ, with the wall a little thicker, 1–1.5μ, and not thickened above, or only moderately so, 2–8μ.

The spirally winged sculpturing of the urediniospores is described in the N. Amer. Flora 7 : 183. The rust, showing uredinia, was detected on a phanerogamic specimen in the Gray Herbarium, on *Rubus Schiedianus* Steud., collected at Coban, Dept. Alta Vera Paz, H. von Türckheim *1149*. It has not yet been taken outside of Guatemala.

14. SPIRECHINA LOESENERIANA (P. Henn.) Arth. (on Rosaceae).
Rubus sp.

A collection secured by C. & E. Seler, at Jalambohoch, Dept. Huehuetenango, August 1896, II, *2687*, was described by P. Hennings under the name *Uredo Loeseneriana*, and this was later made the basis of the genus Spirechina by the writer. Other collections have been made in South America, which supplied telia, but no other collection has yet been reported for North America.

15. KUEHNEOLA MALVICOLA (Speg.) Arth. (on Malvaceae).
Malvaviscus arboreus Cav., Antigua, Dept. Sacatépequez, March 1, 1916, II, III, *543*.
Malvaviscus mollis DC., Huehuetenango, Jan. 22, 1917, II, III, *766*.

A long-cycle rust for which the pycnia are not yet known. It was collected by Kellerman, on *M. arboreus* (host determined by J. Donnell-Smith, Oct. 13, 1911), at Mazatenango, Feb. 28, 1905, II, *5375*, and reported by Kern in Mycologia 3 : 288–290. 1911.

16. PUCCINIASTRUM SPARSUM (Wint.) Ed. Fisch. (on Ericaceae).
Arbutus sp., Cerro Quemado, Dept. Quezaltenango, Jan. 21, 1915, II, *121*.

A heteroecious rust, especially common in western North America. It has not been reported before from so far south. Recent cultures by Dr. Ed. Fischer of Berne, Switzerland, show that in Europe the aecia occur on Picea.

17. MELAMPSORIDIUM ALNI (Thüm.) Kleb. (on Betulaceae).
Alnus acuminata H. B. K., Sololo, 7,500 feet alt., Jan. 29, 1915, II, *150*.
Alnus jorullensis H. B. K., Quezaltenango, Jan. 28, 1917, II, *791*.

Alnus sp., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, *569*.

A heteroecious rust more common northward. It occurs also in Europe. Only uredinia have been seen among North American collections. It was also found by Kellerman at San Rafael, Feb. 3, 1907, on an undetermined Alnus.

18. CEROTELIUM FICI (Cast.) Arth. (on Artocarpaceae).

Ficus padifolia H. B. K., San Felipe, Dept. Retalhuleu, Jan. 13, 1917, II, *707*.

Ficus sp., San Antonio Suchitepequez, Feb. 24, 1916, II, *527;* Retalhuleu, Feb. 26, 1916, II, *536*.

The species was collected by Kellerman on *F. aurea* Nutt., at Gualan, Dept. Zacapa, Jan. 1, 1906, II, *5456*, and reported by Kern in Journ. Myc., *l. c.*

A long-cycle rust, in America known only in the uredinial stage, although very abundant wherever Ficus grows. Some doubt still exists regarding the probable telial form of the American material, and there is a possibility that the generic assignment may not be right. Some mycologists prefer to list it as *Uredo Fici* Cast., or *Physopella Fici* Arth., or *Kuehneola Fici* Butl.

19. CRONARTIUM QUERCUS (Brond.) Schröt. (on Fagaceae).

Quercus sp., San Rafael, Dept. Guatemala, Jan. 10, 1915, II, *56;* Guatemala City, Feb. 14, 1917, II, *866*.

The species was collected by Kellerman on *Q. tomentosa* Willd., at Guatemala City, Feb. 2, 1905, II, *5304*, and reported by Kern in Journ. Myc., *l. c.* Northward the species forms large aecial galls on the branches of pine, but they have not been seen in Guatemala.

20. CRONARTIUM COLEOSPORIOIDES (Diet. & Holw.) Arth. (on Scrophulariaceae).

Castilleja tenuiflora Benth., Solola, 5,300 feet alt., Jan. 27, 1915, II, iii, *125a;* Antigua, Dept. Sacatépequez, Dec. 27, 1916, II, *644;* Quezaltenango, Jan. 28, 1917, II, *788*.

Castilleja sp., Quezaltenango, Jan. 16, 1917, II, *726*.

Lamourouxia cordifolia Schl. & Cham., Guatemala City, Jan. 9, 1917, II, III, *685*.

Lamourouxia dependens Benth., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, *568*.

Lamourouxia rhinanthifolia H. B. K., Quezaltenango, Jan. 21, 1915, II, *101*.

This heteroecious rust is especially abundant in the western mountains of North America. It produces its aecia on the twigs and trunks of various species of pine. Its occurrence on Lamourouxia has not been reported before.

21. CIONOTHRIX PRAELONGA (Wint.) Arth. (on Carduaceae).

Eupatorium morifolium Mill., Guatemala City, Feb. 15, 1916, *491;* same, Jan. 9, 1917, *688;* same, Feb. 8, 1917, *840.*

Eupatorium odoratum L., Mazatenango, Dept. Suchitepequez, Feb. 22, 1916, *525.*

Eupatorium sp., Agua Caliente, Dept. Guatemala, Feb. 10, 1917, *852.*

A short-cycle species. The genus contains forms with telia very similar to those of the long-cycle Cronartium. It was collected by Kellerman on *E. populifolium* H. B. K., at Los Amates, Dept. Izabal, March 15, 1905, *5301*, *5302*, and reported by Kern in Mycologia, *l. c.*

22. ALVEOLARIA CORDIAE Lagerh. (on Ehretiaceae).

Cordia riparia H. B. K., Colomba, Dept. Quezaltenango, Feb. 2, 1917, *821.*

An interesting short-cycle species which probably forms no pycnia. It occurs also in the West Indies and South America.

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A NEW THREE-SALT NUTRIENT SOLUTION FOR PLANT CULTURES

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The majority of the nutrient solutions hitherto employed in the study of the salt nutrition of plants have contained four or more principal salts, besides the trace of a salt of iron, but the 36 different solutions used by Shive[1] differ from the earlier ones in the fact that they all contain the six essential ions (besides iron) in the form of only three salts. These essential ions are: Ca, K, Mg, NO_3, SO_4 and PO_4, and Shive put them into the solution in the form of the three salts, calcium nitrate ($Ca(NO_3)_2$), magnesium sulphate ($MgSO_4$), and mono-potassium phosphate (KH_2PO_4). It is at once suggested that the six requisite ions might enter into the solution as other salts than the three just mentioned, and the question arises whether or not a suitable solution for the growth of plants might not be made with one of the five other possible combinations.

The six logically possible ways by which these six essential ions may enter into the solution, always employing just three salts, are indicated by the following scheme; they are numbered serially, by Roman numerals.

I	II	III	IV	V	VI
$Ca(NO_3)_2$	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$CaSO_4$
KH_2PO_4	K_2SO_4	KNO_3	K_2SO_4	KNO_3	KH_2PO_4
$MgSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Mg(H_2PO_4)_2$	$Mg(NO_3)_2$

Before an adequate discussion of the salt nutritional requirements

[1] Shive, J. W. A study of physiological balance in nutrient media. Physiol. Res. 1: 327–397. 1915. A preliminary announcement appeared as: A three-salt nutrient solution for plants. Amer. Journ. Bot. 2: 157–160. 1915.

[The **Journal** for June (5: 279–336) was issued July 6, 1918.]

of any kind of plant may be possible, all six of these salt combinations must of course be thoroughly tested with reference to the plant in question. Hitherto, only the first of these has received attention in the literature, the salts of group I being those employed in Shive's elaborate study. The present paper shows the results of a preliminary test of a series of solutions using group III—potassium nitrate (KNO_3), magnesium sulphate ($MgSO_4$), and mono-calcium phosphate ($Ca(H_2PO_4)_2$).

In order to investigate in an adequate way the combination of these three salts in plant cultures, it is of course necessary to include a number of different sets of salt proportions and a number of total concentrations or osmotic values, as both Tottingham[2] and Shive have pointed out. Shive tested thirty-six different sets of salt proportions and three different total osmotic values, so that he dealt with 108 different solutions, all made with the same three salts. The preliminary study here reported involved, however, only one total concentration, about the same as the one termed *optimal* by Shive (osmotic value about 1.75 atmospheres), and only twelve sets of salt proportions were here employed. These were selected to correspond to certain ones of Shive's thirty-six, rather evenly distributed over his triangular diagram.

In preparing the solutions, it was assumed as approximately correct that the osmotic effect of dissociation occurring in these solutions is the same for $Ca(H_2PO_4)_2$ as it was taken to be for $Ca(NO_3)_2$ in Shive's work. Likewise, it was assumed that the osmotic effect of the dissociation of KNO_3 in these solutions is approximately like that calculated for KH_2PO_4 by Shive. Finally, it was assumed that the osmotic effect of the dissociation of $MgSO_4$ is the same in our solutions as it was taken to be in Shive's calculations for the same salt. Following these assumptions, the partial volume-molecular concentrations of our salts are the same as those for the corresponding sets of salt proportions shown in Shive's table for the total concentration value of 1.75 atmospheres, but of course we employ $Ca(H_2PO_4)_2$ instead of Shive's $Ca(NO_3)_2$, and KNO_3 instead of his KH_2PO_4. Using Shive's designations for the different solutions (referring to his triangular diagram), the partial volume-molecular concentrations of each of our three salts, in each of our 12 solutions, are given in table 1.

[2] Tottingham, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol Res. 1. 133–245. 1914.

TABLE I

Partial volume-molecular concentrations of each of the three salts used, for twelve different sets of salt proportions, the solution numbers corresponding to those employed by Shive. Total osmotic value about 1.75 atmospheres in every case.

Solution No.	Partial Volume-molecular Concentration		
	KNO_3	$Ca(H_2PO_4)_2$	$MgSO_4$
R1C1	.0036	.0026	.0400
C3	.0036	.0078	.0300
C6	.0036	.0156	.0150
C8	.0036	.0308	.0050
R2C4	.0072	.0104	.0200
R3C1	.0108	.0026	.0300
C6	.0108	.0156	.0050
R4C2	.0144	.0052	.0200
C4	.0144	.0104	.0100
R6C1	.0216	.0026	.0150
C3	.0216	.0078	.0050
R8C1	.0288	.0026	.0050

It was found that all these solutions could be made up from stock solutions of the individual salts, without the formation of precipitate, and that they were stable. Ferric phosphate was added to each culture jar, in the manner followed by Shive.

Wheat of the same variety as was used by Shive ("Fulcaster") was here employed, and the general technique was the same throughout as in his experimentation. Our culture period extended from May 15 to June 2, 1917, thus embracing 18 days. The cultures stood on a rotating table in the same greenhouse as was used by Shive. For the period in question the absolute minimum temperature was 13° C. and the average of the daily minima was 18° C.; the absolute maximum temperature was 39° C. and the average of the daily maxima was 30° C. The corrected water loss from a Livingston standard white spherical porous-cup atmometer was 295 cc. for the period, giving an average rate of 16.4 cc. per day.

Besides the twelve cultures employing the new solutions, our series also included three like cultures with Shive's best solution for wheat, introduced for comparison. This is his solution R5C2 (optimal concentration), containing the following three salts in the partial volume-molecular proportions indicated: KH_2PO_4, 0.0180; $Ca(NO_3)_2$, 0.0052; $MgSO_4$, 0.0150.

The total amount of solution removed from each jar by the six plants during the entire period (which is practically the same as the

amount of water transpired) was determined, as were also the approximate average length of main roots, the dry yield of tops and the dry yield of roots. The sum of top and root yields is of course the total yield.

These data are presented in table 2, which shows the actual values for the three similar Shive solutions, and their average, in each case, and also the *relative* values for each of the twelve new solutions. The relative values have been calculated on the basis of the corresponding average from the Shive solutions, this average considered as unity. Thus, each datum is expressed in terms of the corresponding average from the triplicate Shive solution, and the several data from the new solutions are directly comparable throughout each separate column of the table. The highest value in each column is denoted by bold-face type.

TABLE 2

Water absorbed, average root length and dry yields of tops, of roots, and of entire plants, for wheat grown 18 days in the solutions characterized in table 1, each value expressed in terms of the average of the three corresponding values obtained from Shive's best solution for wheat (controls 1, 2, and 3).

Solution No.	Water Absorbed	Mean Root Length	Dry Yield		
			Tops	Roots	Entire Plants
Control 1	247 cc.	24.6 cm.	.491 g.	.153 g.	.644 g.
" 2	240 cc.	25.1 cm.	.474 g.	.152 g.	.626 g.
" 3	253 cc.	23.5 cm.	.509 g.*	.175 g.	.684 g.
Control average	247 cc.	24.4 cm.	.491 g.	.160 g.	.651 g.
	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
R1C1	.85	.53	.79†	.92	.82
C3	.83	.50	**1.05***	.95	1.03
C6	.57	.28	.70§	.66	.69
C8	.35	.29	.45§	.50	.46
R2C4	.92	.44	.97‡	.83	.94
R3C1	.92	.80	.84†	.99	.88
C6	.48	.30	.58§	.61	.59
R4C2	.97	.66	.95*	.94	.95
C4	.85	.41	.85§	.86	.85
R6C1	1.07	**1.00**	1.02†	**1.14**	**1.05**
C3	1.04	.60	.95‡	.98	.96
R8C1	**1.10**	.94	1.02	1.10	1.04

* Slight magnesium injury.
† Severe magnesium injury.
‡ Slight acid injury.
§ Severe acid injury.

The data of table 2 lead to the conclusion that solution R6C1 is

physiologically the most efficient on the basis of the whole group of criteria employed. Of course this is not to be interpreted to mean that this solution is the very best possible for this plant, for these climatic conditions and for these criteria, since the distribution of our solutions on the triangular diagram is rather open, much more so than in the case of Shive's series of thirty-six different sets of salt proportions, and of course some other set of proportions than those here tested might have given still higher plant values. It is not probable, however, that either the growth values or the salt proportions of such a hypothetical very best solution might have been pronouncedly different from those indicated.

On the triangular diagram, this solution of our series lies very close to Shive's best solution for wheat; ours is R6C1, while his is R5C2, and solution R5C2 was not tested in our series.

The relative data show that our single culture with solution R6C1 gave somewhat higher values than the corresponding average of the three controls with Shive's best solution, in all cases excepting that of root length. The approximate mean length of the main roots for our solution R6C1 is shown to be equal to the control average. The variations due to unknown conditions ("individual variations") are always so great in this sort of experimentation, however, that no particular emphasis should be placed upon the indications that our solution R6C1 gave better growth than did Shive's best; for it is to be remembered that the data for our twelve new solutions are derived from single cultures, of only six plants each. For the present, it is sufficient to say that our three-salt solution R6C1 is apparently *just as good* for the growth of young wheat plants (judged by the numerical data of table 2) as is Shive's optimal R5C2, despite the fact that the proportions of the component ions are considerably different in the two solutions.

It does not seem desirable to enter into further discussion of these results at the present time, on account of the paucity of our data, but it may be of value to present briefly our observations on the apparent health of the plants in the various solutions. The peculiar morphological responses called "magnesium injury" by Tottingham, which frequently occur with Shive's optimal R5C2 for wheat, appeared in some of the cultures (see the asterisks in the fourth column of table 2), and they were pronounced with solution R6C1, the one that appears best on the basis of the numerical values. In this set they occurred

in but one of the three controls. It appears that some evidences of poisoning may be expected whenever maximum dry-weight values are obtained with young wheat plants,[3] if the transpiration rate is not too low. Our best culture *without magnesium poisoning* was with solution R8C1, and this solution appears to be about as efficient as R6C1 on the basis of the growth data of table 2. As might be expected, the greatest water-absorption (transpiration) occurred with solution R8C1, where leaf injury was not manifest, but the difference between R8C1 and R6C1 is not great. In regard to the dry yield of tops, solutions R6C1 and R8C1 appear to be equivalent from the rounded-up data of the table, but there is actually a slight (though insignificant) difference between them, in favor of the former.

Another form of injury, consisting of a withering and darkening of the leaf backward from the tip, occurred in certain cultures that were free from magnesium injury (see those marked ‡ and § in the fourth column of table 2). It is suggested that this is due to too high acidity of the solution, and it may be tentatively called "acid" injury, until the relations of hydrogen-ion concentration are studied in such series as these. No acid injury was manifest with either solution R6C1 or R8C1, nor was it observed in any of the controls.

Summarizing the results of this preliminary study, it appears that the numerical criteria that we employed indicate that our solution R6C1 is as good as (or slightly better than) Shive's optimal solution R5C2. Both these solutions induce some magnesium injury in young wheat plants, however. Considering the occurrence of this injury and the criterion of water absorption, as well as the production of dry yield, the best balanced solution (for these plants) of our entire series is R8C1, which contains, per liter, 0.0288 g.-mol. of KNO_3, 0.0026 g.-mol. of $Ca(H_2PO_4)_2$, and 0.0050 g.-mol. of $MgSO_4$. This solution appears to be better suited to give maximum growth combined with perfect health of plants than does either one of the two other solutions just mentioned. Judging from all the evidence at hand (including

[3] This point was first emphasized by Free and Trelease, who remark: "It may be a general rule that increased growth is the first response to agents or circumstances which would prove injuriously toxic in greater concentration or on longer exposure. . . . In other words, slight poisoning, such as that caused by magnesium or boron, is essential for the production of the greatest dry weight of tops. Either magnesium or boron will serve." See: Free, E. E., and Trelease, S. F. The effects of certain mineral poisons on young wheat plants in three-salt nutrient solutions. Johns Hopkins Univ. Circ. March, 1917, pp. 199–201.

various points brought out in Shive's paper), it appears that our solution R8C1 is physiologically the best balanced for young wheat plants of all the nutrient solutions so far noted in the literature, but this matter assuredly requires still further study.

Of course the proportions of the various atoms and atomic groups (constituting the essential ions) are, in our solutions, very markedly different from the corresponding proportions in Shive's series, and table 3 is here appended to emphasize this aspect of the general problem of the salt nutrition of plants and to put the comparative data thus far available in convenient form for future reference. In this table the solutions are each indicated by the proper Roman numeral (referring to the foregoing scheme of six logically possible series of three-salt solutions) and by the symbol showing the position of the particular set of salt proportions considered, on the triangular diagram employed by Shive. This will furnish a convenient method for future reference to the large number (216) of different solutions that will require attention in this field. All three solutions here considered have approximately the same osmotic value (or total concentration), this value being that employed by Shive for his optimum series (1.75 atm.). The index of total concentration should be most conveniently expressed in terms of the lowering of the freezing-point (the familiar Δ of physical chemistry), but the requisite determinations for a statement of these magnitudes are not yet available. In table 3, solution IR5C2 is Shive's optimal R5C2, while solutions IIIR6C1 and IIIR8C1 are the two new well-balanced solutions brought forward in the present paper.

The first column of table 3 shows the various atoms or atomic groups (ions, whether still in their molecules or free in the solution) with which a physiological discussion of these solutions will eventually have to deal. The third column shows the absolute values of the respective partial concentrations, on the basis of solution volume. These may be called the partial volume-ionic or volume-atomic concentrations, but a new term will be required when the dissociated ions are to be considered separately from those still held in the molecules. To illustrate the significance of these values, the quantity 0.0180 in this column (referring to K) denotes that solution IR5C2 contains 18 thousandths of a gram-atom of potassium in a liter of solution. The same quantity occurs below with reference to H_2PO_4 and also to PO_4, which means that the solution in question contains 18 thousandths of a gram-ion of H_2PO_4 and of PO_4 per liter of solution, the term gram-

ion being used in a sense exactly analogous to that commonly attributed to the corresponding term gram-molecule. It will be noted that the partial volume-atomic concentration value for H is, in this same solution, twice as great as that for K, H_2PO_4, and PO_4, which should be clear from the formula of the salt referred to, KH_2PO_4. The introduction of S and N at the bottom of table 3 does not refer to the existence of such atoms as ions in the solutions, but has reference only to the relative numbers of these atoms present. That these two elements be considered separately in a full description of a nutrient solution seems highly advisable; first, because they are frequently so considered in the literature of nutrient solutions, soil analyses, etc., and second, because it seems highly probable that the supply of these elements to the plant may frequently be important, aside from the particular combinations (compounds or atomic groups) in which they may be supplied. It may be once more emphasized that the use of weight percentages (parts per million, etc.) to designate the partial concentration characteristics of solutions and soils ought to be discontinued in this sort of study, since such terminology is not only meaningless in any thoroughgoing physiological or chemical discussion, but is actually misleading to many readers and clouds the very important issues involved.

The fourth column of table 3 presents the respective values of the third column in relative form, the value given for K in the second column (for the solution R5C2) being here taken as unity throughout. These values thus represent the numbers of the respective atoms, etc., present in each solution, per unit of volume, in terms of the number of potassium atoms in solution IR5C2. It appears, for example, that solution IR5C2 contains 2.89 times as many calcium atoms as it does potassium atoms, per volume unit. The values of the last column of table 3 are likewise relative, but here the partial volume-atomic (or volume-ionic) concentration of the ion or atomic group in question, in solution IR5C2, is taken as unity. As an illustration, it is apparent that there are only half as many atoms of calcium, per unit of volume, in solution IIIR6C1 as there are in solution IR5C2.

The data of table 3 make it clear how very different are the atomic and ionic proportions in Shive's solution IR5C2 and in our best solution, IIIR8C1. The latter can supply to the plant much more potassium and very much more of the nitrate ion than can the former; on the other hand, the supplies of calcium, magnesium, sulphate, and phos-

TABLE 3

Partial volume concentrations of the various atoms and atomic groups (ions) in the three nutrient solutions, IR5C2, IIIR6C1, and IIIR8C1, all three having an osmotic value of about 1.75 atmospheres.

Ion (or Atom)	Solution	Partial Vol.-ionic (or Vol.-atomic) Concentration*		
		Absolute	Relative to	
			K of Same Solution	Same Ion, Etc., of IR5C2
K	IR5C2	.0180	.100	1.00
	IIIR6C1	.0216	.100	1.20
	IIIR8C1	.0288	.100	1.60
Ca	IR5C2	.0052	.289	1.00
	IIIR6C1	.0026	.120	0.50
	IIIR8C1	.0026	.903	0.50
Mg	IR5C2	.0150	.833	1.00
	IIIR6C1	.0150	.694	1.00
	IIIR8C1	.0050	.174	0.33
SO_4	IR5C2	.0150	.833	1.00
	IIIR6C1	.0150	.694	1.00
	IIIR8C1	.0050	174	0.33
H_2PO_4	IR5C2	.0180	.100	1.00
	IIIR6C1	.0052	.240	2.89
	IIIR8C1	.0052	.180	2.89
NO_3	IR5C2	.0104	.578	1.00
	IIIR6C1	.0216	.100	2.08
	IIIR8C1	.0288	.100	2.77
PO_4	IR5C2	.0180	.100	1.00
	IIIR6C1	.0052	.216	2.89
	IIIR8C1	.0052	.180	2.89
H	IR5C2	.0360	.200	1.00
	IIIR6C1	.0104	.481	2.88
	IIIR8C1	.0104	.361	2.88
S	IR5C2	.0150	.833	1.00
	IIIR6C1	.0150	.694	1.00
	IIIR8C1	.005C	.174	0.33
N	IR5C2	.0104	.578	1.00
	IIIR6C1	.0216	.100	2.08
	IIIR8C1	.0288	.100	2.77

* This refers to the atomic group or atom, whether actually separated from the molecule or not; degree of actual ionization is not here considered.

phate are in every case much lower in our best solution than they are in Shive's. Nevertheless, both solutions are excellent for the growth of young wheat plants. As our quantitative and critical knowledge of the relations between nutrient solutions and plant growth is increased, it becomes more and more strongly suggested that it is not atomic, nor

yet ionic nor even molecular, proportions that determine the physiological properties of a solution. The problem thus emphasized is as important in agricultural practice as it is complicated and difficult. The work of many investigators will be required in this field before any serious discussion of the salt-nutrition of plants may even be attempted. It is to be hoped that the results of such work may be allowed to appear as the work goes on, so that our general appreciation of this exceedingly fundamental problem in agricultural science may begin to assume definite form as soon as possible. Thus only can enormous waste of time and human energy, due to our lack of appreciation of these matters, be avoided.

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THE HISTOLOGY OF THE PHLOEM IN CERTAIN WOODY ANGIOSPERMS[1]

L. H. MacDaniels

The study of phloem as a distinct tissue with an important and specialized function may be said to have begun in 1837, when Theodor Hartig discovered the sieve tube and announced that the sieve plates are perforated. This discovery, important as it was, apparently received little recognition for nearly twenty years, when Hartig's observations were confirmed and extended by Von Mohl (1855), Schacht (1860), Nägeli (1861), Hanstein (1865), and Dippel (1869). The work of these men, together with that of De Bary, established the fact of the universal occurrence of the sieve tube in the angiosperms, and of very similar structures in the gymnosperms and the vascular cryptogams.

The work of the last-named author is particularly significant in that, in addition to his own research, the work of previous investigators was brought together and organized and a nomenclature established which, for the most part, is still in use. Since the work of De Bary, numerous investigations on phloem have been published. From the physiological point of view the work of Fischer (1884), Haberlandt (1884), Czapek (1897), and others stands out as important; from the standpoint of histology, probably the most significant publications are those of Wilhelm (1880), Janczewski (1882), Russow (1883), Lecomte (1889), Poirault (1893), Perrot (1899), Strasburger (1901), and two papers by A. W. Hill (1901, 1908). These papers vary much in nature, but are concerned chiefly with the development and structure of the phloem of mature stems. The idea of relating anatomy and histology to taxonomy and phylogeny by the methods followed by Schwendener and Solereder, and, in a broader and more fundamental way, by Williamson and other workers in paleobotany, had not been applied to the phloem by any of these investigators. The papers of Jeffrey, however, in 1900 (11) and 1902 (12), laid re-

[1] Contribution from the Department of Botany, College of Agriculture, Cornell University.

newed emphasis on the value of anatomy in taxonomy, maintaining that internal morphology should be given weighty consideration in working out phylogenetic relationships. Thus, mainly upon anatomical characters, the division of vascular plants into two great groups, the Lycopsida and the Pteropsida, has been made. In these papers Jeffrey has also put forth and strongly emphasized the importance in plants of the principle of recapitulation based on the fact that the seedling in its ontogeny often recapitulates ancestral characters. Hence, by the study of sporelings of pteridophytes and seedlings of angiosperms, stelar types in these groups and the probable evolution of the stele have been determined.

In addition to types of stele, various other characters have also been given weight in classification. Among these may be mentioned types of vessel and the distribution of parenchyma in xylem. It is held by the last-named author and others (6) that vessels with scalariform end walls are primitive, whereas those with porous ends are advanced. In the same way, the terminal position of the wood parenchyma is considered most primitive, the vasicentric position most advanced, and the diffuse position intermediate (9).

Phylogenetic significance was first assigned to types of sieve tubes found in angiosperms by A. F. Hemenway in two papers entitled "Studies on the Phloem of the Dicotyledons" (1911, 1913). In the first of these, covering six species of the Juglandaceae, the point is made that the sieve tubes of that family have well-developed sieve plates upon the side walls which do not differ from those on the end walls. Such a condition resembles that found in the gymnosperms and vascular cryptogams; hence the phloem of the Juglandaceae is primitive, and its sieve tubes represent the type primitive among angiosperms.

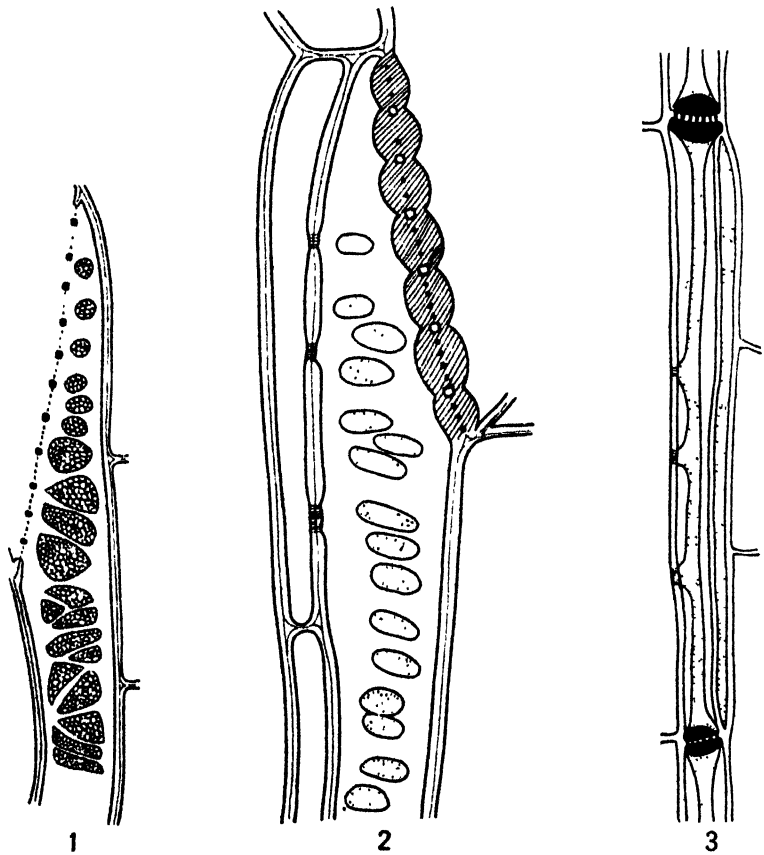
In Hemenway's second paper, after the study of ninety-six species of woody dicotyledons belonging to seventy-five genera and enough herbaceous monocotyledons and dicotyledons to make the total number of genera studied one hundred and forty, the following points are made:

The forms studied are grouped under three principal types which are indicated in text-figures 1–3, after Hemenway's figures.

The first type, according to that author, resembles the sieve tube of Pinus, with the sieve plates on the side wall identical with those on the end wall. The terminal wall in this case is very oblique, extending from one fourth to one half the length of the sieve tube element.

This type is shown in text-figure 1, representing the sieve tube of *Juglans nigra.*

"The second type is like the first except that the lateral sieve plates are less well developed and the end walls less oblique, having two to ten sieve plates each. This type may be shown by Vitis" (text-fig. 2). The sieve plates are shown covered with callus.



TEXT-FIGS. 1–3. Sieve tube segments are shown cut longitudinally in the tangential plane so that the sieve plates on the end walls are seen in section and those on the side walls are seen face view. (See text.)

The third type has the end wall made up of a single sieve plate which is placed nearly at right angles to the side walls. The sieve

plates, or lattices, on the side walls are not so well developed as in the other types. This condition is illustrated by *Lactuca scariola* (text-fig. 3).

In these three types there is a correlation between the type of side wall and the type of end wall; type 1 has well-developed lateral plates, whereas type 3, the highest type, has the lattices poorly developed.

Thirty species of the lower woody dicotyledons were studied and found to have the same general sieve tube structure as have the gymnosperms and vascular cryptogams. In general there is no wide variation in type of sieve tube even in different genera of the same family, except when there are both herbaceous and woody genera within the family, in which case the herbaceous plants show the higher type.

No woody dicotyledons studied have sieve tubes of the third type. In the list of species arranged in groups according to type, the woody dicotyledons are placed almost exactly in the phylogenetic order of the Engler and Prantl system. The herbaceous dicotyledons and the monocotyledons are all placed above the second type.

Companion cells are rare if not wanting in many of the lower dicotyledons.

The evolution of the sieve tube parallels that of the vessel, there being a gradual transition from the gymnosperm type with oblique end walls and well developed lateral sieve plates to the so-called dicotyledonous type with transverse sieve plates and poorly developed plates or lattices upon the side walls.

The study of sieve tube types adds an argument in favor of the view that herbaceous plants are more advanced in evolutionary development than woody plants.

The initial purpose of the present research was to make a study of the phloem of seedlings of a number of woody dicotyledons selected to represent a series from those phylogenetically lowest to those highest in the Engler system. Such a study was expected to reveal any differences between the structure of the phloem in the seedling and in the mature plant, both root and stem, and to supply evidence of recapitulation. If the latter were present, it was hoped that light would be thrown on what constitutes the primitive type of sieve tube among angiosperms. As the work progressed, however, discrepancies of a rather startling nature began to appear between the results of the present research and those of Hemenway. Further, examination of

literature showed that there are also many published descriptions of sieve tubes of woody dicotyledons which do not agree with Hemenway's classification. This led, in so far as time has allowed, to a checking of Hemenway's results in the woody dicotyledons by the careful examination of many of the species described by him. This has been taken up in addition to the seedling study originally planned.

The following material has been examined. Collections marked N. S. followed by the age, indicate material taken from a branch of that age on a mature tree. Mature phloem with few exceptions was taken during the dormant season from thrifty trees ten inches or more in diameter. Growing material was also taken from mature trees. Mature root material was obtained from the larger roots of mature trees.

LIST OF MATERIAL STUDIED

Acer Negundo L.
Mature, twig.
Acer rubrum L.
Growing, twig.
Acer saccharum Marsh.
Mature, growing, mature root, 5-yr. stem, 1-yr. stem.
Aesculus Hippocastanum L.
Mature, twig.
Ailanthus glandulosa Desf.
Mature, N. S. 5-yr., mature root, growing, 5-yr. stem, 5-yr. root, 1- to 2-yr. stem, twig.
Alnus incana (L.) Moench.
Mature.
Benzoin aestivale (L.) Nees.
Mature, twig.
Betula lutea Michx. f.
Mature root, mature, 3-yr. stem, twig.
Carya cordiformis (Wang.) K. Koch.
Mature, 3-yr. stem.
Carya glabra (Mill.) Spach.
Mature root, 4-yr. stem, 3-yr. root, twig.
Carya ovata (Mill.) K. Koch.
1-yr. stem, 1-yr. root.
Castanea dentata (Marsh.) Borkh.
Mature.
Catalpa bignonioides Walt.
Mature, twig.
Celtis occidentalis L.
Mature.
Cephalanthus occidentalis L.
Mature, mature root, growing, 2-yr. stem, 4-yr. root, twig.
Cornus alternifolia L. f.
Mature.
Cornus Amomum Mill.
2-yr. stem, 2-yr. root.
Cornus paniculata L'Her.
Mature root.
Eleagnus angustifolia Pursh.
Growing.
Fagus grandifolia Ehrh.
Mature, twig.
Fraxinus americana L.
Mature, mature root, growing, 6-yr. stem, 6-yr. root, 2-yr. stem, 2-yr. root, 1-yr. twig.
Fraxinus nigra Marsh.
Mature, twig.
Gleditsia triacanthos L.
Mature.
Gymnocladus dioica (L.) Koch.
Twig.
Juglans cinerea L.
Mature, growing, mature root, 1-yr. stem, 1-yr. root, twig.
Juglans nigra L.
Mature, N. S. 10, 8, 6, 4, 2-yr., 4-yr. stem, 4-yr. root, 2-yr. stem, 2-yr. root.
Liriodendron Tulipifera L.
Mature, mature root, 10- to 12-yr. stem, 10- to 12-yr. root, 3-yr. stem, 3- to 4-yr. root, 3-yr. root, twig.
Maclura pomifera (Raf.) Schneider.
Mature.
Magnolia acuminata L.
Mature, mature root, 12-yr. stem, 5- to 7-yr. root, sucker shoot, 2-yr. twig.
Morus alba L.
Twig, mature.

Nyssa sylvatica Marsh.
Mature, twig.
Ostrya virginiana (Mill.) K. Koch.
Mature.
Platanus occidentalis L.
Mature stem, mature root, growing, 4-yr. stem, 4-yr. root, 2-yr. stem, 2-yr. root, twig.
Populus deltoides Marsh.
Mature, mature root, growing, 4-yr. stem, 4-yr. root, 3-yr. stem, 3-yr. root, 1-yr. stem, 1-yr. root.
Prunus Persica (L.) Stokes.
Mature.
Prunus serotina Ehrh.
Mature.
Pyrus communis L.
Mature.
Pyrus Malus L.
Mature old tree, mature young tree, growing, mature root, 5-yr. stem, 5-yr. root, twig.
Quercus alba L.
Mature, mature root, growing.
Quercus bicolor Willd.
Twig.
Quercus rubra L.
3-yr. stem, 3-yr. root.
Rhamnus alnifolia L'Her.
Twig.
Rhus typhina L.
Mature, growing, mature root, 3-yr. stem, 3-yr. root, 1-yr. stem, 1-yr. root, twig.
Ribes americana Mill.
2-yr. twig.
Robinia Pseudo-Acacia L.
Mature, growing, mature root, 4-yr. stem, 4-yr. root, 2-yr. stem, 2-yr. root, 1-yr. stem, 1-yr. root, twig.
Salix alba var. *vitellina* (L.) Koch.
Mature.
Salix nigra Marsh.
Mature, growing, mature root, 2-yr. stem, 2-yr. root.
Sambucus canadensis L.
Mature, growing, mature root, 3-yr. root.
Sambucus racemosa L.
1-yr. stem, 1-yr. root, twig.
Sassafras variifolium (Salisb.) Kuntze.
Root of small tree, 2-yr. twig.
Tilia americana L.
Mature, 3-yr. stem, 3-yr. root, 2-yr stem, 2-yr. root, 1-yr. root, twig.
Ulmus americana L.
Mature stem, mature root, growing, 5- to 6-yr. stem, 5- to 6-yr. root, 3-yr. stem, 3-yr. root.
Ulmus fulva Michx.
Mature.
Viburnum Lentago L.
Mature, growing, mature root, 6- to 10-yr. root.

METHODS

A saw and a chisel were used in obtaining material from the trees. This was cut into approximately one-centimeter cubes including phloem and contiguous cambium and xylem, and killed in chrom-acetic acid, 0.75 percent–1 percent. It was then desilicified by placing it in hydrofluoric acid, one half commercial strength, for two weeks; dehydrated through a series of alcohols; embedded in celloidin; sectioned on a sliding microtome; stained in Delafield's haematoxylin and safranin, and mounted in balsam. It was found that collections made in the early fall soon after cambial growth stopped were usually in better shape than those made in the early spring before development began, because in many species the sieve tubes were badly crushed at the latter time.

The description of material is taken up in phylogenetic order. For the sake of brevity, many of the descriptions are omitted and the descriptions themselves are abridged. For convenience, the following outline is followed for the description of each species.

Sieve tubes:[2] Size; abundance; distribution; type of end wall—*i. e.*, type 1, very oblique with 10–20 sieve plates separated by scalariform bars; type 2, with oblique end wall with 2–10 sieve plates between scalariform bars; and type 3, with a single plate, either transverse or slightly oblique; lattice-description (by lattice is meant the sieve fields on the side walls of the sieve tubes).

Companion cells: abundance; distribution.

Parenchyma: distribution; abundance; type, whether "cambiform" (*i. e.*, elongated), prosenchymatous, or "conducting" (*i. e*, shorter, larger in diameter, and thin-walled, usually in series vertically. These terms are after Haberlandt, 4).

Salix nigra

Mature. Sieve tubes abundant, in irregular tangential rows between bands of fibers, not collapsed even in older portions; end walls oblique, of first type, with 8–15 sieve plates; lattice with fine pores between cellulose bands, almost identical with that of *Populus deltoides* (fig. 7); pores in sieve plates 2–3 μ in diameter, those in sieve fields of lattice about 0.4–0.5 μ.

Companion cells in the corners of about one third the sieve tubes, sometimes extending the entire width.

Parenchyma of one type, the divided cambiform; for the most part filled with dark-staining granules; heavily pitted on end and radial walls with sieve-like pits; cells about equal to sieve tubes in number; in tangential bands along the bands of fibers and scattered among the sieve tubes.

Growing. No new features except that fewer parenchyma cells are filled with tannin[3] in the current year's growth.

Mature root. Differs from mature stem only in having somewhat less sclerenchyma (fig. 8).

Two-year stem from rapidly growing plant. Sieve tubes few in number and small as compared with mature conditions; most elements with oblique end walls, a few with transverse sieve plates; on oblique end walls the plates are more widely separated than in mature phloem; lattice not well developed; walls of tubes apparently much thinner than in mature sieve tubes; actual breadth of sieve plates 10–20 μ as contrasted with 20–50 μ in mature.

Companion cells present in normal numbers.

Parenchyma of same type as mature.

Populus deltoides

Mature stem. Phloem identical with that of *Salix nigra* except in minor details. Smaller proportion of parenchyma filled with dark-staining granules than in Salix.

[2] Although in its best usage the term sieve tube refers to a series of cells joined end to end (see De Bary, 2), for convenience the term is here also used to designate a single element or cell unit of a sieve tube.

[3] In this paper the term *tannin* is used to signify the dark brown cell content that is abundant in many plants. In many cases it is chemically tannin, but in others it is organic material of similar appearance.

Width of sieve plates 30–45 μ. Width of sieve pores 3.5–5.5 μ (fig. 2). Pores on side walls mere dots not over 0.5–0.6 μ, not so distinct and clear cut as in sieve plate (fig. 3).

Mature growing and *mature root* show no new features in phloem except reduction of sclerenchyma in latter (fig. 7).

Four-year stem. Rapid growth (fig. 6). Sieve tubes abundant, same type as mature but smaller, width of sieve plates 15–25 μ; plates not so crowded on terminal wall as in mature; lattice not well developed.

Companion cells in normal numbers.

Parenchyma abundant, of two types: one short, wide, usually filled with tannin, the other elongate, heavily pitted on the radial walls and not containing tannin.

Four-year root. Same as four-year stem except for increase in storage tissue and reduction of amount of sclerenchyma; all parenchyma full of starch.

Three-year stem. Slow growth (fig. 5). Same general features as in four-year stem but number and size of sieve tubes greatly reduced; lattice not well developed; width of sieve plates 12–20 μ.

Companion cells present.

Tissue packed with starch; length of sieve tubes less than in mature.

Three-year root. Sieve tubes smaller and fewer than in stem of same age. Pores on sieve plates not well developed; lattice not well developed, none observed; storage tissue abundant, packed with starch. Fewer sieve plates on end wall than in older material.

One-year stem (fig. 4). Sieve tubes very small and scarce, much more so than in the three-year material, difficult to find; some sieve plates transverse, usually only two or three plates on oblique walls. Width of sieve plates 6–10 μ; parenchyma full of starch.

One-year root. Same in general as one-year stem. Sieve tubes fewer in proportion to parenchyma; some with transverse walls in same section with those with as many as six sieve plates on an oblique end wall.

Juglans nigra

Mature (fig. 15). Phloem very much like that of Salix and Populus in general features. Sieve tubes abundant, scattered among parenchyma, of lowest type, with very oblique end walls, sometimes having as many as 20 sieve plates; lattice well developed, much as in Carya (fig. 9) but not so regular; pores visible but not more than 0.5–0.6 μ in diameter; pores on sieve plates 1.8 to 3.5 μ, as in Populus (figs. 2, 3).

Companion cells not abundant, probably present in about one sieve tube out of ten.

Parenchyma of divided cambiform type, frequently filled with dark staining substance.

N.S. 12 to N.S. 2. Phloem taken from the top at points at 12, 10, 8, 6, 4, and 2 years of age showed gradual decrease in size of sieve plate from 25–40 μ in width to 12–20 μ in the two-year material; also a reduction in number of sieve tubes as compared with the number of parenchyma cells, a reduction very similar to that observed in a series of seedlings of different ages.

Four-year stem (fig. 14). Sieve tubes small, 6–18 μ; few in proportion to parenchyma cells; lattice not well developed.

Companion cells rare, about as abundant as in mature.

Parenchyma of divided cambiform type; some cells longer than others.

Four-year root. Same as the four-year stem except more storage tissue; sieve tubes in groups separated by wide rays

Two-year stem (fig. 13). Shows further reduction in size and number of sieve tubes and increase in proportional amount of parenchyma.

Two-year root. Same as the two-year stem except for increased amount of storage tissue.

Juglans cinerea

Mature, growing and *mature root* same as *Juglans nigra* except that latter has more fibers in phloem.

One-year stem (fig. 12). Sieve tubes very small and scarce; sieve plates 8–12 μ in width; lattice not apparent; oblique end wall with several plates with poorly developed pores; companion cells not found but probably present; parenchyma abundant, some cells elongated and conspicuously pitted, others short and packed with starch

Carya cordiformis

Mature (fig. 9). Sieve tubes single or in groups of 3 or 4 surrounded by single row of parenchyma cells, each strand of sieve tubes and parenchyma embedded in an almost solid mass of fibers; sieve tubes with very oblique end wall, with 10 to 20 sieve plates (fig. 9); lattice very regular, cross-bars separating sieve fields; pores of sieve fields very minute, pores in sieve plates on end walls 3–4.5 μ in diameter.

Companion cells present in corners of sieve tubes, difficult to determine

Parenchyma of divided cambiform type, abundantly pitted on radial walls, confined to sieve tubes or in narrow tangential bands; many cells filled with tannin.

Carya ovata

One-year stem, one-year root. Same as above-described species in essential features; sieve tubes small; 3 to 4 sieve plates on oblique end walls, plates rather widely separated; lattice very faint.

Companion cells probably present.

Parenchyma heavily pitted and abundant.

Fagus grandifolia

Mature. Sieve tubes large with single transverse sieve plates 50–60 μ across; pores on sieve plates large, 6–9 μ in diameter, pores on lattice distinct but small, about 0.5 μ; lattice not well developed.

Companion cells not easily seen on account of condition of material.

Parenchyma scattered, not abundant.

Castanea dentata

Mature. Sieve tubes in rather small numbers as compared with parenchyma; end walls oblique with 4–8 large sieve plates; lattice about as in Populus.

Companion cells rare.

Parenchyma of two types, one with protoplasmic contents, sometimes tannin, the other brick-shaped, apparently empty, with conspicuous sieve-like pitting on all walls especially the radial and terminal. (This type distinct and unusual.)

Ulmus americana

Mature (figs. 16, 17). Sieve tubes collapsed except close to cambium; sieve plates transverse with large meshes, 4–7 μ in diameter; lattice with prominent cross-bars and conspicuous pores (fig. 17); pores of lattice about 0.6–0.8 μ in diameter.

Companion cells frequent.

Parenchyma of two types: one large and tannin-filled, arranged in tangential rows; the other divided-cambiform, heavily pitted on radial walls, scattered among the sieve tubes.

Growing. No new features except reduction in amount of sclerenchyma.

Five- to six-year stem. Sieve tubes very small and scarce; of the same type as those of the mature stem; lattice not well developed.

Parenchyma as in mature but a proportionally larger amount in relation to sieve tubes.

Three-year root. Sieve tubes very rare, difficult to find; sieve plates transverse as in mature, but poorly developed; sieve tubes of about the size of some of the parenchyma cells.

Parenchyma abundant, thin-walled.

Maclura pomifera

Mature (fig. 18). Sieve tubes short and broad with transverse sieve plates in tangential bands between bands of heavily pitted parenchyma; lattice not well developed.

Companion cells fairly common.

Parenchyma of two types; one heavily pitted on radial and transverse walls, the other thin-walled, containing crystals; these arranged in bands in the following succession: 4–5 rows of heavily pitted parenchyma, 1–2 rows of crystal-containing parenchyma, 2–4 rows of sieve tubes.

Morus alba

Mature (fig. 19). Sieve tubes abundant, short, rather wide, with a single transverse sieve plate; lattice forming a distinct network as in Ulmus; pores in lattice distinct.

Companion cells in about one half of sieve tubes.

Parenchyma of divided cambiform type, heavily pitted on radial wall; not filled with tannin; scattered among sieve tubes and in irregular tangential bands.

Liriodendron Tulipifera

Mature (fig. 21). Sieve tubes of type 2, with 6–10 sieve plates upon an oblique end wall; lattice of rounded, well-defined sieve areas on both tangential and

radial walls, most abundant on radial; side walls of younger tubes completely covered with a layer of bluish-staining substance, probably callus. Companion cells abundant in the corners and across the sides of the tubes.

Parenchyma not abundant; of divided-cambiform type, heavily pitted among the radial walls.

Mature root (figs. 22–25). Differs from mature stem only in having less sclerenchyma.

Three-year stem. Phloem areas small, between large fan-shaped rays. Sieve tubes small and few as compared with amount of parenchyma; sieve plates transverse in majority of cases; lattice not apparent.

Parenchyma of two types, the elongate phloem parenchyma distinct from the storage type.

Three-year root. Differs from stem of same age only in having no fibers and in having smaller amount of phloem as compared with storage tissue.

Twig. Sieve tubes small with sieve plates either on an oblique end wall or along sides of tubes.

Parenchyma of same type as in mature.

Sassafras variifolium

Root of small tree. Sieve tubes not abundant; single sieve plates transverse or slightly oblique; lattice not well developed.

Companion cells not observed on account of collapsed condition of sieve tubes

Parenchyma of divided-cambiform type; heavily pitted upon radial walls.

Benzoin aestivale

Mature. Sieve tubes small, nearly occluded with callus-like substance; sieve plates single, either transverse or oblique; lattice not conspicuous.

Companion cells present in about one half of the sieve tubes.

Parenchyma of divided-cambiform type, partially filled with tannin-like substance; frequent large secretory cells.

Platanus occidentalis

Mature. Sieve tubes crushed except next cambium; large with oblique end walls; type 2, with 3–10 large plates 35–45 μ wide, frequently separated rather widely; lattice in the form of small pore areas upon both radial and tangential walls; no well-defined cross bars as in Populus.

Companion cells present in about one third of sieve tubes.

Parenchyma of divided-cambiform type, frequently filled with tannin; heavily pitted on the radial, and to some extent on the tangential walls.

Four-year stem. Phloem small in amount, in restricted areas between rays; sieve tubes small with transverse end plates, or plates upon side wall; plates small, 8–12 μ in width; lattice not apparent on uniformly thin side walls.

Four-year root. Like stem of same age.

Two-year stem. Sieve tubes showing sieve plates widely separated upon side walls; width of plates 6–8 μ.

Parenchyma elongated with abundant pitting.

Two-year root. Like stem of same age.

Pyrus Malus

Mature (*old tree*). Sieve tubes abundant, long and narrow with no clearly defined end wall (fig. 27); radial walls covered with closely set sieve plates sometimes in two rows (fig. 26); plates of different sizes; pores in different plates of nearly the same size, 0.8–1.5 μ, only a few with smaller pores; no sieve plates on the tangential walls.

Companion cells in about one half the sieve tubes.

Parenchyma of two types: one, the elongate-cambiform with well-developed protoplasts; the other longer, containing abundant crystals.

Prunus Persica

Mature (fig. 28). Sieve tubes next cambium filled with a blue-staining callus-like substance as in Benzoin, very narrow with end walls usually as in type 3; occasional end walls with more than one plate, but this exceptional; lattice not observed.

Companion cells not discernible with certainty.

Parenchyma of slender cambiform type, partially occluded as are the sieve tubes.

Gleditsia triacanthos

Mature. Sieve tubes short, with oblique end wall, with 4–10 narrow sieve plates; tubes full of mucilage or protoplasm; lattice not apparent.

Companion cells not discernible because of heavily staining sieve tube contents.

Parenchyma of elongate divided-cambiform type.

Robinia Pseudo-Acacia

Mature (figs. 30, 36). Sieve tubes of type 1, without lattice; sieve plates 35–40 μ in width.

Companion cells abundant in corners of sieve tubes.

Parenchyma of broad conducting type, thin-walled with abundant pitting.

Mature growing (figs. 31, 32). Formation of rows of parenchyma and of sieve tubes from cambium cells of same length. Otherwise no new features.

Mature root. Differs from mature stem only in having reduced amount of sclerenchyma.

Four-year stem. Sieve tubes of same type as mature but much smaller; small aggregations of slime near center of each; sieve plates poorly developed, 10–15 μ in width; lattice not apparent.

Companion cells present in most of the sieve tubes.

Parenchyma very abundant; heavily pitted on the radial walls.

Four-year root. Very similar to stem of same age but with more storage parenchyma and less sclerenchyma.

Two-year stem. Much as in four-year material, but fewer sieve tubes as compared with parenchyma; sieve tubes very small, with poorly developed plates 7–10 μ in width.

Parenchyma very abundant; packed with starch.

Two-year root (fig. 35). Like stem of same age but more storage tissue.

One-year stem, one-year root. Sieve tubes very small with poorly developed sieve plates; would not be recognizable except for dark-staining slime; very similar to parenchyma in size and shape.

Ailanthus glandulosa

Mature (fig. 29). Sieve tubes collapsed except close to cambium; large, very thin-walled, with usually a single transverse sieve plate; rarely 2–3 sieve plates. Pores of sieve plate very large; lattice not present.

Companion cells probably present but not observed on account of condition of material.

Parenchyma of conducting type; very thin-walled, in strands accompanying the sieve tubes; a second type of parenchyma with rounded ends forming loose tissue between sieve-tube groups

Mature growing, mature root. Showed no different features.

Five-year stem. Sieve tubes small but with single transverse plate; pores in plate frequently few.

One- to two-year stem. Sieve tubes of type 3, the same as in mature; very rare.

Parenchyma of elongate cambiform type.

Rhus typhina

Mature. Phloem tissue uniformly thin-walled; sieve tubes abundant, with 1–5 sieve plates upon an oblique end wall; frequently only a single plate; lattice not well developed.

Companion cells abundant in corners of tubes.

Parenchyma of conducting type, very thin-walled; cells about the same size as the sieve tubes.

Three-year stem. Sieve tubes abundant but forming smaller proportion of phloem than in the mature; end walls either transverse or oblique with one plate or several; sieve plates rarely on side walls of tubes; lattice not seen.

Parenchyma the same as in mature

Three-year root. The same as stem except for the presence of more storage tissue filled with starch.

One-year stem, one-year root. Essentially the same as in three-year material; fewer sieve tubes in proportion to parenchyma.

Acer saccharum

Mature. Sieve tubes with a single transverse sieve plate in the majority of cases, frequently with 2–3 plates; lattice weak, a faint network of cellulose thickenings upon the side wall; pores of lattice distinct under the oil immersion objective.

Companion cells not readily distinguished because of condition of material.

Parenchyma of divided-cambiform type in irregular tangential bands.

Tilia americana

Mature (fig. 39). Sieve tubes of type 2 with 2–10 sieve plates; rarely a single transverse plate; lattice well developed, much as in Populus.

Companion cells abundant in corners of sieve tubes.

Parenchyma of two types: one narrow, divided-cambiform type heavily pitted on radial wall; the other large, thin-walled cells of conducting type, but nearly empty or containing crystals; both types in tangential bands.

Three-year stem, three-year root. Sieve tubes in small groups between large fan-shaped rays; small, few, of same type as mature.

Parenchyma of two types as in mature.

One-year root. Sieve tubes not found; cambiform and storage parenchyma abundant.

Two-year twig. Sieve tubes about the same size and type as in three-year seedling; more numerous in twig.

Eleagnus angustifolia

Mature growing (fig. 20). Sieve tubes of type 3 with somewhat inclined sieve plates; lattice much as in Ulmus.

Companion cells very abundant in corners of sieve tubes.

Parenchyma of short, broad, divided-cambiform type; thin-walled.

Cornus paniculata

Mature root (figs. 37, 38). Sieve tubes small, slender; usually with a single transverse sieve plate; sometimes with 2–4 plates upon an oblique end wall; lattice a fine network enclosing pores much as in Acer.

Companion cells present but not abundant.

Parenchyma of divided-cambiform type with conspicuous sieve-like pitting on radial and terminal walls.

Nyssa sylvatica

Mature. Sieve tubes very long with oblique end walls; type 1 with 4–15 plates; plates in many cases present over entire radial wall where in contact with another sieve tube; frequently separated rather widely; lattice a very faint network with distinct pores in the meshes.

Companion cells present but not abundant.

Parenchyma scattered among sieve tubes; short divided-cambiform, full of protoplasm; abundant pitting on radial walls.

Twig. Sieve tubes present with transverse sieve plates for most part; very thin-walled, resembling parenchyma cells but with easily recognized sieve plates.

Parenchyma of very elongate, divided-cambiform type.

Fraxinus americana

Mature. Sieve tubes about the same shape as cambium cells; type 2 with 3–6 plates; pores large; thin-walled; lattice not well developed.

Companion cells abundant in corners of sieve tubes.

Parenchyma of broad, divided-cambiform type; abundant, scattered among sieve tubes; very heavily pitted on radial walls.

Two-year stem, two-year root, fast growth. Sieve tubes small, few, thin-walled, with single poorly developed transverse sieve plates; lattice not observed.

Parenchyma as in six-year stem.

One-year twig. Sieve tubes small, thin-walled, resemble parenchyma. Much like two-year seedling.

Fraxinus nigra

Mature. Sieve tubes of type 3, the single plate either transverse or somewhat oblique; pores in plate large; lattice a faintly developed network with fine pores.

Companion cells not observed on account of crushed condition of sieve tubes.

Parenchyma abundant; of divided-cambiform type, not very heavily pitted.

Cephalanthus occidentalis

Mature root (fig. 40). Sieve tubes long and narrow, with one or two round sieve plates either transverse or oblique; lattice not well developed.

Companion cells abundant.

Parenchyma, scattered cells of the conducting type, heavily pitted.

Sambucus canadensis

Mature (figs. 43, 44). Sieve tubes few, small, with oblique end walls, 10–20 plates; 20–30 μ in width, sometimes rather widely separated on the side walls; lattice not well developed.

Companion cells present in most of sieve tubes.

Parenchyma very abundant; short cambiform type, heavily pitted laterally.

Three-year root (fig. 42). Sieve tubes small and few, of same type as mature; plates 8–18 μ in width.

Parenchyma full of starch, as are all cells of xylem except vessels; not conspicuously pitted.

Sambucus racemosa

One-year stem (fig. 41). Sieve tubes small and scarce; 1–5 sieve plates on end wall.

Companion cells present.

From the foregoing descriptions of material it becomes evident that there are certain differences and certain similarities between the conditions found in the phloem of seedlings and in that of mature plants. Among the similarities, perhaps the most significant observation, from the standpoint of the present research, is that apparently there is no fundamental difference between the type of sieve tube found in seedlings and that found in the mature condition. Species having sieve tubes with transverse sieve plates in the mature plant show the same type in the seedling, *e. g.*, Robinia, Ailanthus, and Ulmus. In plants with sieve tubes of types 1 and 2, such as Populus, Juglans, Liriodendron, and Sambucus, the similarity between the sieve tubes of seedlings and adults is apparent, though not so marked, in that the seedlings of these plants show sieve tubes with a smaller number of sieve plates and frequently with a single transverse plate. Such a reduction in number of plates might perhaps be taken to indi-

cate that the single transverse plate is the primitive condition. It seems more probable, however, that the small number of sieve plates in the sieve tubes of seedlings is due to the greatly reduced size of these elements in the young plants. If such is the case, the significance of the small number of sieve plates in the sieve tubes of seedlings of plants with the first and second types of sieve tubes in the adult, would be physiological rather than phylogenetic.

The extent of the differences in size between the sieve tubes of the seedling and those of the adult is readily shown by the following series of measurements of the radial width of sieve plates upon the end walls of sieve tube elements and by the series of photomicrographs.

Populus: one-year stem, fig. 4, 6–10 μ; three-year stem, fig. 5, 12–20 μ; four-year stem, fig. 6, 15–25 μ; mature, fig. 7, 30–45 μ.

Juglans: one-year stem, fig. 12, 8–12 μ; four-year stem, fig. 14, 6–18 μ; mature, fig. 15, 25–40 μ.

Robinia: two-year root, fig. 35, 7–10 μ; four-year root, 10–15 μ; mature, fig. 36, 35–40 μ.

Sambucus: three-year root, fig. 42, 8–18 μ; mature, fig. 43, 20–30 μ.

Since a single sieve plate extends completely across the sieve tube radially, its measurement may be taken as an accurate indication of the width of the tube. The difference in length between the sieve tubes in seedlings and those in mature plants is usually apparent also, but is not so marked. In fact, such difference in length is present in the cambium cells themselves, as is shown by figures 31 and 33 which are of like magnification. The first of these is taken from the tangential section of the cambium of a four-year seedling, whereas the second comes from a similar position in a mature stem.

These figures show that in passing from the one-year seedling to the adult, there is a gradual increase in the size of the sieve tube. Such small size as that indicated for the younger seedlings would make the presence of the adult number of sieve plates on the end wall impossible, without extreme reduction either of the size or of the number of pores upon the sieve plate. As a matter of fact, such reduction in size and number of pores does occur but not to the extent necessary for the accommodation of so large a number of plates on an end wall.

The small size of the sieve tubes in seedlings is correlated with the small number of these elements that are present in phloem of young plants, as compared with the number of parenchyma cells. This is graphically shown in Populus (figs. 4, 5, 6), Juglans (figs. 12–14),

Robinia (fig. 35), and Sambucus (figs. 41, 42). It is further shown in the two figures of Robinia (34, 32), which are taken from tangential sections of the phloem near the cambium in a four-year seedling and a mature tree, respectively. Figure 34 shows that the cambium is developing almost entirely into parenchyma cells, whereas figure 32, representing the condition of a homologous portion of the phloem of the mature plant, shows the formation of an abundance of sieve tubes in addition to the parenchyma.

No seedlings were found in which the number of sieve tubes as compared with the number of parenchyma cells was not much smaller than in the mature plant. In fact, in some of the one-year seedlings this condition was so extreme that no sieve tubes could be found even after long search with the oil immersion lens (*e. g.*, Tilia and Robinia). This, of course, does not mean that sieve tubes are entirely absent, but it does at least indicate that they are very rare.

In many of the younger seedlings examined, as indicated throughout the description, the few sieve tubes that were found were poorly developed. The sieve tubes themselves were very small (see figs. 4, 5, 12, 35); the lattice was very faint or not discernible, and the sieve plates were frequently either without distinct pores or with very minute pores, and were not placed with any regularity upon the walls. In some cases the sieve tube elements were so much like parenchyma in size and shape that they could not easily be distinguished. For example, in the two-year seedlings of Robinia ready recognition of the sieve tubes would be impossible, were not red-staining slime strands present in these elements.

The phloem of the one-year-old twigs of mature plants shows many of the features found in seedlings, although they are not so extreme. Thus the first annual ring of phloem generally shows poorly developed sieve tubes without lattice and sometimes without well-developed sieve plates. The sieve tubes are fewer in number, as compared with the number of parenchyma cells, than in the adult, and a greater proportion of the sieve plates are transverse. The size of the sieve plates, also, is much smaller than in the mature plant, as shown in *Juglans nigra*. Here there is an increase in the size of sieve plates from 12–20 μ in the two-year twig to 25–40 μ in a twelve-year-old branch.

The structure of the phloem of seedlings as indicated in the foregoing observations, especially as regards the small number and poor development of the sieve tubes and the abundance of well-developed

parenchyma, strongly suggests the possibility that in seedlings the conduction of protein material may take place, in large part, through the heavily pitted parenchyma. Of course it cannot be stated with certainty that such is the case, for it cannot be assumed that the few sieve tubes present in seedlings are not sufficient to supply the necessary conduction. Yet it seems reasonable to suppose that in a thrifty seedling considerable protein must be conducted through the phloem in excess of the apparent accommodation for such conduction through sieve tubes. It is well known that certain proteins may be conducted through parenchyma, as in the central cylinder of some of the mosses, the endings of the vascular bundles in leaves, and in the leptome parenchyma, discussed by Haberlandt (4, p. 329). The structure of seedling phloem suggests that such may be the case there also, and that the sieve tube, being a highly complex and specialized structure, is not well developed until later in the ontogeny of the plant.

In comparing the phloem of roots with that of stems, no essential differences are found in type of sieve tubes. This fact has been previously brought out by Russow (16, p. 205). The phloem of the root shows minor differences from that of the stem, in that in the latter there is usually a greater amount of sclerenchyma. In the material examined, the only exception to this condition is the root of *Viburnum Lentago*, where the masses of sclerenchyma in the root were considerably larger than those in the stem. Another point to be brought out is that the phloem of roots, particularly that of seedlings, shows a greater abundance of storage parenchyma differing from ordinary phloem parenchyma in that the cells are more nearly globose. In some seedlings practically all the phloem tissue is composed of cells of this sort. This condition may be so extreme as to give the impression that sieve tubes are fewer in number in seedling roots than in stems of the same age. Such seems to be the fact in a number of species, but as the observations lack the confirmation of actual count and measurement, it cannot be so stated with certainty.

The great variation in the type and distribution of sclerenchyma in phloem tissue, a condition of course comparatively well known, is clearly brought out in this study. All conditions may exist from a nearly complete absence of sclerenchyma, as in Maclura and Rhus, to practically solid masses of fibers and stone cells making up the entire older phloem of Fagus and Platanus. In the latter type all the phloem tissue, except that actually functioning, soon becomes converted into,

or completely crushed by, sclerenchyma. Fibers may be scattered singly throughout the phloem as in Cephalanthus (fig. 40), arranged in regular tangential bands as in Salix and Tilia (fig. 39), or placed in irregular groups. They may be fairly straight, as is usually the case, or may be very much contorted and twisted as in *Acer rubrum*. Stone cells may occur singly or, as is more common, may be grouped in irregular masses. Both fibers and stone cells are frequently grouped together in various ways. The distribution of sclerenchyma is distinct for a given species, and within a species is fairly constant as far as the number of collections concerned in this study show.

The origin of fibers and stone cells in the phloem seems to be very different in different plants. In many cases fibers are cut off by the cambium in regular tangential bands and therefore appear near the cambium. Just as frequently, however, sclerenchyma is not present except in the outer phloem, in which case it is formed either by the lignification of existing parenchyma cells formed by the cambium, the proliferation of parenchyma and its subsequent lignification, or possibly by the formation of rows of sclerenchyma by a layer of cells comparable to a phellogen layer. Frequently the medullary rays are lignified along with the masses of parenchyma through which they pass.

Crystals are of very frequent occurrence in the phloem of many plants. Probably the rhomboidal type is the most common, occurring abundantly within stone cells and septate fibers and also in different types of parenchyma. Druses are frequent but usually within parenchyma cells rather than sclerenchyma. The type and distribution of crystals, however, seems to vary with ecological conditions and rapidity of growth.

The parenchyma in the phloem of the mature woody plants studied is rather surprisingly uniform, the divided-cambiform type of Haberlandt (4) or modifications of it being of almost universal occurrence. The true cambiform type of cell with prosenchymatous form was not found in any of the plants studied, and the so-called conducting type was found in only a few, *e. g.*, Tilia and Cephalanthus. In fact, from the material studied, it seems practically impossible to make a good distinction between the cambiform and the conducting types because of the intergrading forms, a point brought out by Strasburger (17). In the development of secondary phloem parenchyma, an elongate cambium cell (figs. 10, 31) divides longitudinally to produce a parenchyma mother cell, which in turn divides transversely to form the

parenchyma cells proper. This gives rise to a vertical series of cells with the cell at each end of the series pointed, as was the cambium from which it was formed (figs. 11, 32). Parenchyma of the true cambiform type would be produced either by the failure of the parenchyma mother cell to divide transversely or through its division by oblique cross walls so that resulting cells would be prosenchymatous in shape. Such division, however, was not observed, the cross walls being nearly transverse in all cases. The divided-cambiform type shows great variation in shape and size, from elongate, narrow cells with little radial depth, to nearly cubical cells approaching the conducting type.

The pitting in the divided-cambiform parenchyma is in most cases very prominent on the radial and terminal walls. Frequently the pits are grouped so as to present a sieve-like appearance as shown in the parenchyma of *Cornus paniculata* (figs. 37, 38), *Castanea dentata*, and others. In some species the pitting is not so pronounced, but the entire walls of the cell are thin and probably allow rapid diffusion. These types of parenchyma, according to Haberlandt (4), serve in the conduction of carbohydrates and the more easily diffusible proteins, a function for which they are apparently well suited histologically.

As before indicated, the present study does not confirm the phylogenetic significance of sieve-tube type as maintained by Hemenway (6). In making such a statement, of course, it must be borne in mind that in evolutionary development morphological characters do not advance equally. A given group may thus have one character highly developed and yet possess others remaining at a low level, as, for example, the presence of the inferior ovary, a "high" character, in "low" families such as the Juglandaceae and the Hydrocharitaceae. It must be recognized, also, that the number of species in this research is perhaps too small, as compared with the vast number of woody plants in the world flora, to justify the making of any sweeping generalizations. It is particularly unfortunate, too, that there are not included representatives of the tropical floras where woody plants form so large a proportion of the whole. Yet, inasmuch as the material examined was selected to represent, in so far as possible, a series of woody plants from the lowest to the highest forms in the Engler system, and since references in literature to the phloem of tropical woody plants show that the condition found there is not different from that in the

northern flora, it seems not unreasonable to suppose that the facts revealed in this research give at least a fairly accurate indication of the conditions present in woody plants as a whole.

In view of the facts brought out in this study and confirmed in literature, it cannot be said that there is definite evidence of gradual advance in the evolution of the sieve tube from type 1 to type 3; at least, if there is such an advance it does not in any way parallel our present ideas of phylogeny. Indeed, Hemenway does not appear to be justified in making his types for sieve-tube classification as indicated by his figures (shown earlier in this paper), because in that classification the oblique end wall with many sieve plates is correlated with well-developed lattice, or sieve plates upon the side walls and the transverse end wall bearing a single plate with poorly developed lattice. From the description of material it is evident that no such correlation exists. For example, both *Ulmus americana* (fig. 17) and *Eleagnus angustifolia* (fig. 20) have single transverse sieve plates, and at the same time well-developed lattice. A similar condition was also pointed out in *Fagus sylvatica* by Dippel (3, p. 255), and later for *Ficus elastica* by De Bary (2, p. 177). Cases where the end walls are oblique and the lattice not well developed are even more numerous, *e. g.*, *Nyssa sylvatica*, *Alnus incana*, *Catalpa bignonioides*, *Fraxinus americana*, and others. Here the end walls are very oblique with from four to fifteen plates, whereas the lateral sieve fields are not clearly differentiated, the side walls being almost without cellulose thickenings and closely set with pores.

Table 1 shows in a graphic way that the sieve tube does not undergo a transition from type 1 to type 3 throughout the series of families as they are now placed in the evolutionary scale. It represents a partial list of the genera examined, arranged in phylogenetic order. Sieve tubes are classified according to the number of sieve plates on the end walls without reference to the side walls. As previously stated, number 1 indicates the lowest type with many sieve plates upon the end wall and number 3 the highest type with a single transverse plate. It is, of course, understood that such a definite classification cannot be actually made since all manner of intergrading forms exist. Under vessel types the scalariform, or lowest, is referred to by number 1, the porous, or highest, by number 3, and those forms which have both scalariform and porous vessels by number 2. Under parenchyma distribution the lowest, or terminal, position is indicated by number 1,

TABLE I

	Sieve Tube Type	Vessel Type	Wood Parenchyma Distribution
Salicaceae			
Populus deltoides	1	3	1*
Salix nigra	1	3	3
Juglandaceae			
Juglans cinerea	1	3	2
Carya cordiformis	1	3	2
Betulaceae			
Ostrya virginiana	1	3	2
Betula lutea	2	1	2
Alnus incana	1	1	2
Fagaceae			
Fagus grandifolia	3	3	2
Castanea dentata	2	3	2
Quercus alba	2	3	2
Urticaceae			
Ulmus americana	3	3	3
Celtis occidentalis	3	3	3
Maclura pomifera	3	3	3
Morus alba	3	3	3
Magnoliaceae			
Magnolia acuminata	2	2	1*
Liriodendron Tulipifera	2	1	1*
Lauraceae			
Sassafras variifolium	3	2	3
Benzoin aestivale	3	3	3
Platanaceae			
Platanus occidentalis	2	2	2
Rosaceae			
Pyrus Malus	1	3	2
Prunus serotina	2	3	2
Prunus Persica	3	3	2
Leguminosae			
Gleditsia triacanthos	2	3	3
Robinia Pseudo-Acacia	3	3	3
Simarubaceae			
Ailanthus glandulosa	3	3	3
Anacardiaceae			
Rhus typhina	2–3	3	3
Aceraceae			
Acer saccharum	2–3	3	3
Acer Negundo	2–3	3	3
Sapindaceae			
Aesculus Hippocastanum	2	3	3
Tiliaceae			
Tilia americana	2	3	2
Eleagnaceae			
Eleagnus augustifolia	3	3	2
Cornaceae			
Cornus paniculata	2–3	1	2
Nyssa sylvatica	1	1	2

* In the starred species the position of the parenchyma is thought to be reduced condition rather than a truly primitive one (9).

TABLE 1—*Continued*

	Sieve Tube Type	Vessel Type	Wood Parenchyma Distribution
Oleaceae			
Fraxinus americana	2	3	3
Fraxinus nigra	3	3	3
Bignoniaceae			
Catalpa bignonioides	2	3	3
Rubiaceae			
Cephalanthus occidentalis	2–3	3	2
Caprifoliaceae			
Viburnum Lentago	1	1	2
Sambucus canadensis	1	2	1

the diffuse, or intermediate, position by number 2, and the highest, or vasicentric, position by number 3.

It will be noted in table 1 that there is considerable discrepancy between the classification of material in this study and that given by Hemenway. That author, as before stated, places all the woody dicotyledons in the two lower types and lists seventy-five genera of woody plants, according to sieve-tube type, almost exactly in phylogenetic order. That such an arrangement is not true to fact is shown beyond question by the present research, and further, is amply confirmed by an examination of literature. Of the species of woody plants placed in types 1 and 2 by Hemenway, the following species were found to have sieve tubes distinctly of type 3, the highest type; *Fagus grandifolia*, *Ulmus americana*, *Celtis occidentalis*, *Maclura pomifera*, *Morus alba*, *Sassafras variifolium*, *Robinia Pseudo-Acacia*, and *Ailanthus glandulosa*. The transverse nature of the sieve plates of Ulmus, Maclura, Morus, Robinia, and Ailanthus is shown in figs. 16, 18, 19, 30, and 29 respectively. Further confirmation of the above-cited determinations is given in the case of Fagus and Maclura by De Bary (2), Ulmus, Morus, and Ailanthus by Lecomte (13), and Robinia by Strasburger (17).

An idea of the extent to which the transverse sieve plates are found in woody plants is shown in the list given in table 2, compiled from Russow (16), Lecomte (13), De Bary (2), Janczewski (10), and Strasburger (17). The names are arranged in phylogenetic order and each is followed by the initial of the author referring to it.[4]

[4] These same authors also make frequent reference to woody plants having sieve tubes with oblique end walls. As this is recognized to be a common type, however, a compiled list has been omitted to economize space.

TABLE 2

Piperaceae
 Piper Cubeba (R.)
Fagaceae
 Fagus sylvatica (D.J.)
Ulmaceae
 Ulmus effusa (L.)
 U. montana (R.)
Moraceae (L.)
 Maclura (D.)
 Ficus macrophylla (R.)
 F. stipulacea (R.)
 F. carica (R.D.)
Artocarpaceae (L.)
Berberidaceae (L.)
Anonaceae
 Anona Chierimolia (R.)
Ranunculaceae
 Atragene (R.)
Rosaceae
 Spiraea (L.)
 Rosa (L.R.J.)
 Amygdaleae (L.)
Leguminosae
 "Papilionaceae" (L.) (R.)
 Cytisus (R.)
 Robinia (S.)
 Halimodendron (R.)
Simarubaceae
 Ailanthus glandulosa (L.)
Malpighiaceae (L.)
Buxaceae
 Buxus (L.)
Coriariaceae
 Coriaria (R.)
Anacardiaceae
 Pistacia (L.)
Aquifoliaceae
 Ilex aquifolium (R.)
Rhamnaceae (L.)
Vitaceae
 Vitis canescens (L.)
Sterculiaceae (L.)
Cornaceae (L.)
Oleaceae
 Fraxinus excelsior (R.)
Asclepiadaceae
 Asclepias (L.)
Verbenaceae (L.)

It is readily seen by a study of tables 1 and 2 that transverse sieve plates are present in many genera of woody dicotyledons without reference to their phylogenetic position. Not only is there no gradual transition in type from the lowest to the highest, but further, in many families and even in some genera, the type of sieve tube is not constant. High and low types of sieve tubes follow each other without any apparent correlation. Thus in Fagus and the Urticaceae, where low types might be expected, we find the single transverse sieve plate to be of universal occurrence, whereas in the Caprifoliaceae the sieve tube is definitely of type 1.

Variation in sieve-tube type within families is in some cases very striking. For example, in the Fagaceae the genus Fagus stands out as belonging to type 3, among genera of lower type. In the Cornaceae, the genus Nyssa has sieve tubes of type 1, whereas Cornus has sieve tubes almost entirely of the third type.

The woody Rosaceae and Leguminosae are additional examples. In the former, *Pyrus Malus* and *Prunus serotina* show sieve tubes of types 1 and 2 respectively, whereas *Prunus Persica* has sieve tubes of type 3 with few exceptions (fig. 28). In the tribe Caesalpinoideae of the Leguminosae type 2 prevails in *Gleditsia triacanthos* and *Gymnocladus dioica*, but in the tribe Papilionoideae type 3 is the rule, as

shown in *Robinia Pseudo-Acacia* (fig. 30) and indicated in literature for Cytisus and Halimodendron (16).

In contrast with the families that show striking variation of sieve-tube type there are also those which show unusual uniformity. Thus the Salicaceae, Juglandaceae, and Betulaceae show almost universally the presence of sieve tubes of type 1, as shown both in the present research and in literature. The Urticaceae also, as indicated in tables 1 and 2, show the third type of sieve tube as a constant character. If sieve-tube type has any phylogenetic significance, it might well be argued that this family belongs higher in the scale of evolution than it now stands.

Sieve-tube type within the genus seems to be constant in the great majority of cases. Certain exceptions do occur, however, even in the rather small number of plants that have been examined. Perhaps the most glowing example of variation within a genus is shown in Fraxinus. Here *Fraxinus americana* has, beyond question, sieve tubes with oblique end walls as in Tilia, but *Fraxinus nigra*, as shown in this study, and *Fraxinus excelsior*, as shown by Russow (16), have sieve tubes with transverse end walls. In the genus Prunus, *Prunus serotina* has sieve tubes of the second type but *Prunus Persica* has those of type 3. This latter case is perhaps not so much to be wondered at because the two species are not closely related within the genus. An indication of this condition is shown in literature by Lecomte (13), who lists *Vitis canescens* as belonging to type 3, when it is well known that the "vine type" of phloem, as in *V. vinifera*, *V. labrusca*, and *V. vulpina*, has the oblique end wall. So far as has been determined, sieve-tube type is constant within a species. In the present study several collections of a species have been made in many cases, the material being chosen from different individuals and from various habitats, and in no instance has any essential discrepancy been apparent.

In view of the evidence brought out in tables 1 and 2, there seems to be no basis for the statement of Hemenway (6) that the study of the sieve tube adds further evidence to the theory that herbaceous plants are more advanced in evolutionary development than is the woody type. This theory regarding the position of herbaceous plants is doubtless well grounded; the point emphasized here, however, is that the so-called "herbaceous type" of sieve tube is of such common occurrence among woody plants that on the basis of our present knowledge there seems to be little foundation for the statement that the

study of the sieve tube either adds to, or subtracts from, the evidence that herbaceous plants are highly advanced in the evolutionary scale.

Another statement by the last-mentioned author (6), which is not confirmed by the present research, is to the effect that companion cells are very rare, if not wanting, in many of the lower woody dicotyledons. It is, of course, realized that the companion cell is often difficult of certain recognition, even in the best preparations. However, if small nucleated cells containing neither starch nor "tannin" are present in the corners or along the sides of the sieve tubes, giving the appearance of having been cut off from the sieve tubes by division, it seems reasonable to conclude that such cells are companion cells. Cells of this type were found in fair numbers in all the material studied except in a few species in which the sieve tubes were so badly crushed that companion cells could not be recognized with certainty The presence of companion cells in woody plants belonging to the lower families is shown quite plainly in the photomicrographs of the transverse sections of *Populus deltoides* (fig. 7), *Juglans nigra* (fig. 13), and *Liriodendron Tulipifera* (fig. 22). Further, the presence of companion cells in Salix and Populus is described by Strasburger (17, pp. 211, 214), who also states that in Salix the companion cell may extend across the entire radial width of the sieve tube. It is evident, however, that these cells do not occur in the lower woody dicotyledons in such abundance as in the more highly placed families, as shown in *Tilia americana* (fig. 39), *Cephalanthus occidentalis* (fig. 40), and *Sambucus canadensis* (fig. 43).

On looking for correlation of sieve-tube type with type of vessel and wood parenchyma distribution, as indicated in table 2, it becomes evident that from the study of so small a number of species no marked relationships can be said to exist. For example, in the lower Amentiferae the lowest type of sieve tube is present in the same plants with the porous vessel and, in the case of *Salix nigra*, with the vasicentric type of parenchyma. In the genus Pyrus, also, sieve tubes of type 1 are associated with porous vessels and diffuse parenchyma. Examples of the association of the high type of sieve tube with the scalariform type of vessel are not so numerous, *Cornus paniculata* being the only good example noted.

In fact, although correlation between these structures does not stand out, it may be said that a fair percentage of cases shows sieve tubes, vessels, and parenchyma of approximately the same level of development according to types made. The large number of cases

where no correlation exists is perhaps within the limits to be expected for morphological characters. It can also be said that the distribution of sieve-tube type in the phylogenetic sequence is apparently just as consistent with the idea that type 1 is primitive and type 3 advanced, as is the distribution of vessel type with the more widely accepted theory that the scalariform vessel is primitive, and the porous advanced; and if the types of vessel are given phylogenetic significance, sieve-tube types may also be given equal value. It cannot be stated, however, that there is a gradual advance in type of sieve tube without great variation or many exceptions in ascending the phylogenetic tree, as indicated by Hemenway (6).

The above-named author also appears to be unjustified in the statement that the sieve tubes of the Juglandaceae are like those of the gymnosperms and vascular cryptogams (5). The reason given for such an assertion is that the sieve tubes of the Juglandaceae have well-developed sieve plates upon the side walls which do not differ in structure and appearance from those upon the end walls, a condition similar to that present in the above-mentioned groups. In a later paper (6) it is stated that thirty species of the lower woody dicotyledons show the same sieve-tube structure as that found in the Juglandaceae.

These statements regarding the sieve tubes of the Juglandaceae and the "thirty species of lower dicotyledonous trees" are confirmed neither by the present research nor by literature. In the first place, as stated by Hill (7), the sieve plates of the gymnosperms, as illustrated by Pinus, have multiperforate sieve fields between the meshes of the cellulose framework, whereas in the angiosperms, as illustrated by Cucurbita, Wisteria, Vitis, and others, the sieve fields between the meshes of the sieve are perforated by a single large pore which takes up nearly the whole width of the mesh. In the present research, examination of the sieve plates and the lattice of the sieve tubes of Juglans, Salix, Populus, and others under high magnification, shows that there is such a discrepancy in the size of the pores on the lateral and terminal wall that they cannot be said to have the same structure. In all cases the pores on the sieve plates of the end walls are large enough to permit accurate measurement, but on the side walls the pores, though distinct, are so minute as to prevent such measurement even under the oil immersion. This condition is well brought out in the photomicrographs of *Populus deltoides* (figs. 2, 3). In fig. 2 the pores in the sieve plate on the end wall are plainly visible, but on the

side wall the pores are so minute that they could not be recorded by the camera, being mere crowded points of light even under high magnification. The sieve plates and lattice of the Salicaceae studied are of nearly identical type with those found in the Juglandaceae. The difference in the size of the pores in the sieve plates and in the lattice is further shown by the measurements given in table 3.

TABLE 3

Juglans nigra	1.8–3.5 microns	.5–.6 micron
Populus deltoides	3.5–5.5 "	.5–.6 "
Salix nigra	2–3 "	.4–.5 "

If similarity to the gymnosperm type of sieve tube is found anywhere in the angiosperms, it apparently is not in the Juglandaceae but rather in such genera as Pyrus and Sambucus, which do show sieve plates of like structure upon both the side and end walls (figs. 26, 27, 44).

In conclusion, it may be said that the present research is not thought to refute the idea that sieve-tube type and type of vessel have phylogenetic significance. Either to confirm or to disprove those theories would require the examination of a vastly greater number of genera representing the floras of the world. Rather is it desired forcibly to bring out the fact that the evolutionary significance of any character is often obscure and that sweeping generalizations cannot be made upon the examination of a limited amount of material, even though the observations may be accurate. That histological and anatomical characters will have a very important influence upon the future ideas of phylogeny cannot be doubted. In the case of most characters, however, it remains for anatomical work on a scale much greater than that hitherto attempted to show what the real significance of those characters may be.

SUMMARY

The more important results of this study may be briefly stated as follows:

1. In the woody dicotyledons, there is no fundamental difference in type between the sieve tubes in the phloem of seedlings and those in the mature plant, existing differences being mainly those of size.

2. In the phloem of seedlings the number of sieve tubes as compared with the number of parenchyma cells is much smaller than in mature phloem.

3. Phloem of one-year-old twigs from mature trees has many of the characteristics found in the phloem of seedlings.

4. There is no gradual advance in sieve-tube type from type 1 to type 3 which parallels our present ideas of phylogeny.

5. All types of sieve tubes are present in the woody dicotyledons, the so-called "herbaceous type," with single transverse sieve plates, being probably about as frequent as any other.

6. Widely different types of sieve tubes are found in the woody species of the same family and sometimes even within the woody species of the same genus.

7. Companion cells are present in all families of the woody dicotyledons studied, although usually in smaller numbers than in the phylogenetically higher forms.

8. The sieve tubes of the lower woody dicotyledons are fundamentally different from those of the gymnosperms and vascular cryptogams.

9. There is little correlation between type of vessel and type of sieve tube.

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EXPLANATION OF PLATES XXIV–XXIX

The following abbreviations are used in labeling the figures: st = sieve tube, sp = sieve plate, l = lattice or lateral sieve field, cc = companion cell, p = parenchyma, c = cambium, f = fibers, m = medullary ray. All figures are photographs of phloem except when otherwise designated.

Pinus Strobus

FIG. 1. Radial section showing distribution of sieve plates upon the radial walls of the sieve tubes. × 250.

Populus deltoides

FIG. 2. End wall of sieve tube showing perforations in the sieve plates. × 450.

FIG. 3. Side wall of sieve tube showing the lattice. The pores between the scalariform bars are very minute as compared with those in the sieve plates on the end wall. × 450.

FIG. 4. Transverse section, one-year-old root showing the small size and number of sieve tubes as compared with the mature condition (fig. 7). × 100.

FIG. 5. Transverse section, three-year-old stem showing the same features as figure 4. × 100.

FIG. 6. Transverse section, four-year stem showing same features as figure 4. × 100.

Populus deltoides

FIG. 7. Transverse section, mature root. Note increase in size and number of sieve tubes in series (figs. 4–7). × 100.

Salix nigra

FIG. 8. Tangential section, mature root, showing end walls of sieve tubes with sieve plates in section. The phloem of Salix and that of Populus are practically identical. × 100.

Carya cordiformis

FIG. 9. Tangential section, mature stem, showing lattice in face view and sieve plates in section. × 100.

Juglans cinerea

FIG. 10. Resting cambium, mature plant, showing shape of cells in tangentia section. × 100.

FIG. 11. Tangential section taken in the phloem near the cambium showing division of cambium cells to form parenchyma and sieve tubes. The sieve-tube elements are of the same length as the cambium cells. × 100.

FIG. 12. Transverse section of one-year stem showing small sieve tubes and abundant parenchyma. × 100.

Juglans nigra

FIG. 13. Transverse section of two-year root showing increase in size and number of sieve tubes as compared with one-year material (fig. 12). × 100.

FIG. 14. Transverse section of four-year stem showing increase in size and number of sieve tubes as compared with two-year material (fig. 13). × 100.

Juglans nigra

FIG. 15. Transverse section of mature stem. Note series (figs. 12–15). × 100.

Ulmus americana

FIG. 16. Radial section showing transverse sieve plates with slime strings adhering to them. × 250.

FIG. 17. Tangential section showing sieve tube with transverse sieve plate and well-developed lattice. × 270.

Maclura pomifera

FIG. 18. Radial section of mature stem showing transverse sieve plates. × 250.

Morus alba

FIG. 19. Radial section showing transverse sieve plates. × 270.

Eleagnus angustifolia

FIG. 20. Tangential section showing single, transverse, or oblique sieve plates and lattice. × 250.

Liriodendron Tulipifera

FIG. 21. Mature stem, transverse section. Sieve tubes in lower part near cambium are partially occluded with callus. × 100.

FIG. 22. Mature root, transverse section. Note much smaller amount of sclerenchyma as compared with stem. × 100.

FIG. 23. Radial section showing large sieve plates in face view upon the end walls and smaller well-developed plates on the side walls. × 180.

FIG. 24. Radial section, mature root, showing variation in size of sieve plates upon end and side walls. × 200.

FIG. 25. Same, tangential section showing sieve plates in section and close contact of sieve tubes, parenchyma, and medullary rays. × 125.

Pyrus Malus

FIG. 26. Radial section showing sieve plates upon radial walls of sieve tubes. × 450.

FIG. 27. Same, tangential section showing sieve plates in section. In this species there is apparently no differentiation of side and end walls. × 270.

Prunus Persica

FIG. 28. Mature stem, tangential section showing single transverse oblique sieve plates. × 450.

Ailanthus glandulosa

FIG. 29. Mature stem, transverse section showing single transverse sieve plates in face view. × 450.

Robinia Pseudo-Acacia

FIG. 30. Mature stem, radial section showing transverse sieve plates, slime globules and probable companion cells. × 270.

FIG. 31. Resting cambium of mature stem, tangential section. × 100.

FIG. 32. Tangential section of phloem near the cambium showing development of cambium cells into parenchyma and sieve tubes. × 100.

FIG. 33. Cambium of four-year stem, tangential section showing smaller size of cambium cells as compared with the mature (fig. 31). × 100.

FIG. 34. Phloem near cambium of four-year stem, tangential section. × 100.

FIG. 35. Transverse section, two-year root. Note small number and size of sieve tubes as compared with mature (fig. 36). × 270.

Robinia Pseudo-Acacia

FIG. 36. Mature growing stem, transverse section. × 270.

Cornus paniculata

FIG. 37. Transverse section showing sieve-like pitting in end walls of parenchyma. × 450.

FIG. 38. Radial section showing sieve-like pitting on radial walls. × 450.

Tilia americana

FIG. 39. Transverse section, mature stem. Note two types of parenchyma. × 100.

Cephalanthus occidentalis

FIG. 40. Transverse section, mature stem. × 100.

Sambucus canadensis

FIG. 41. Transverse section, one-year root. × 270.

FIG. 42. Transverse section, three-year root. × 270.

FIG. 43. Transverse section, mature stem. × 270.

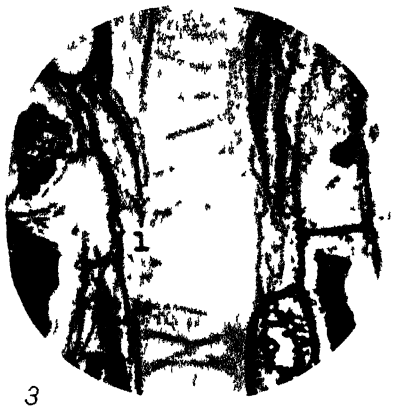
FIG. 44. Tangential section showing sieve plates in section upon oblique end walls. × 270.



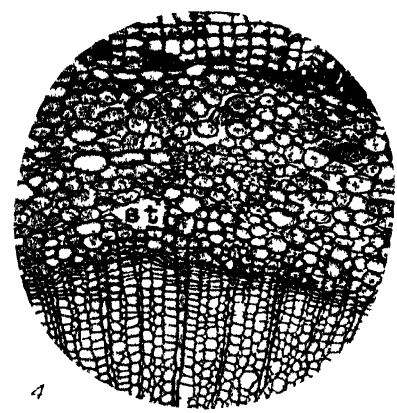
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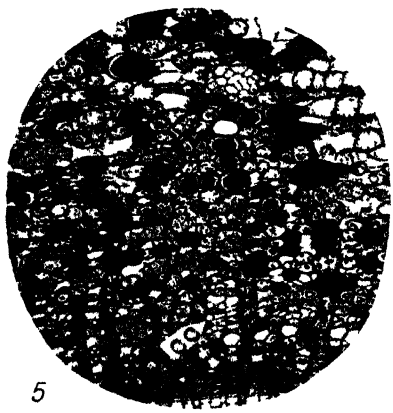
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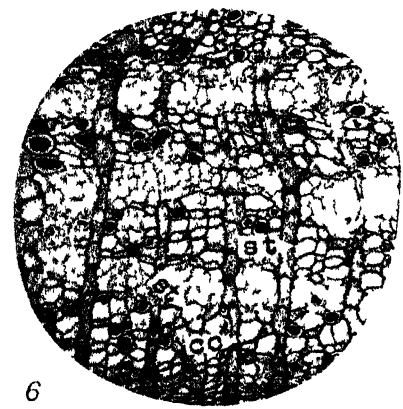
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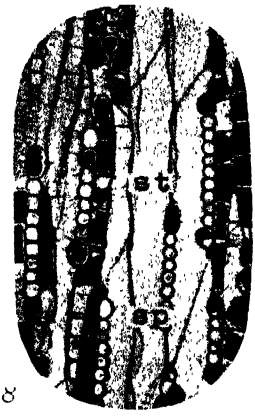
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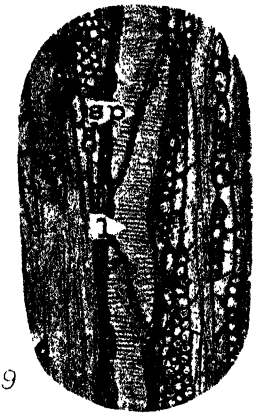
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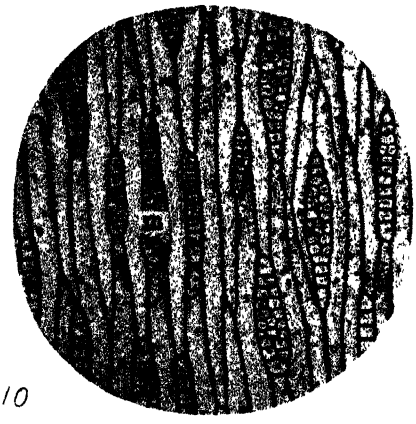
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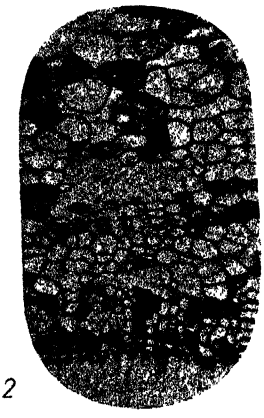
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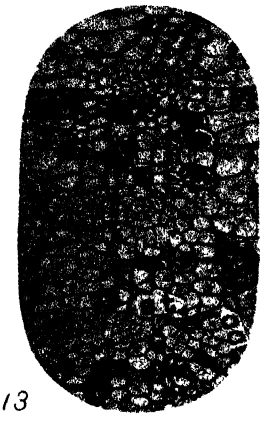
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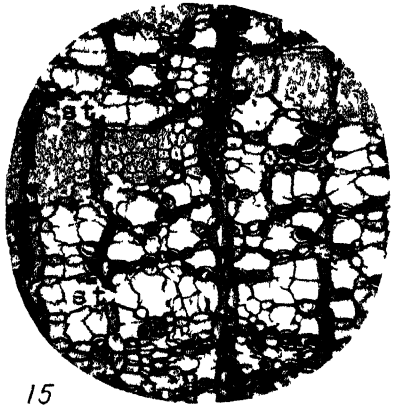
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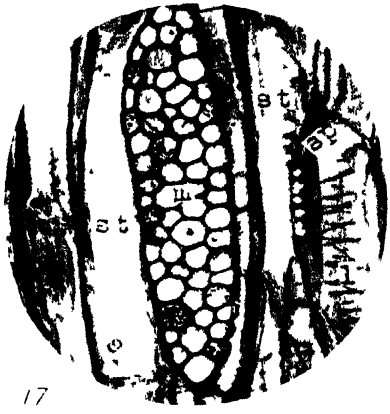
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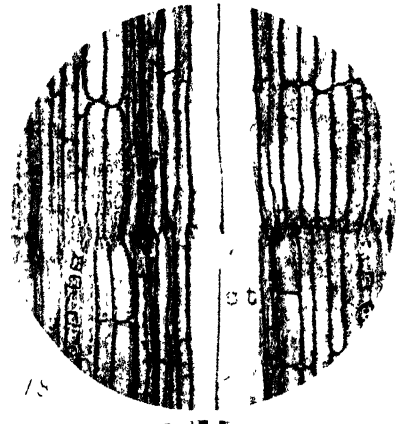
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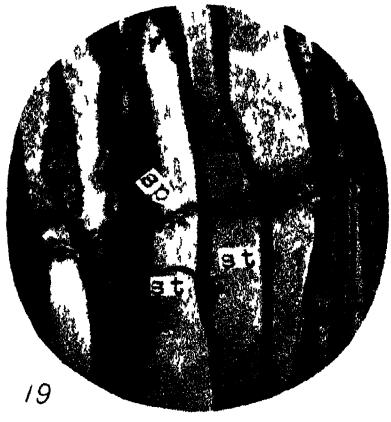
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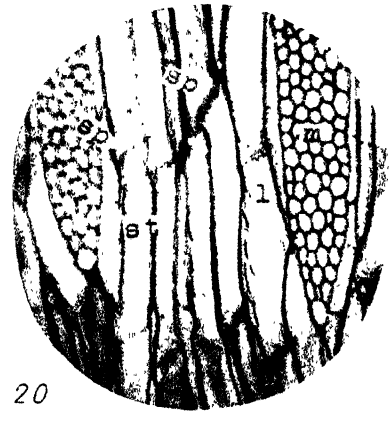
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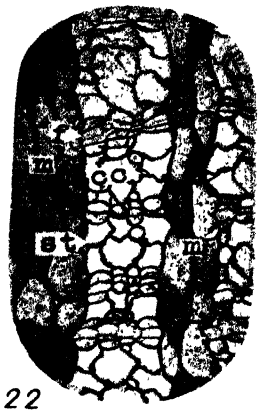
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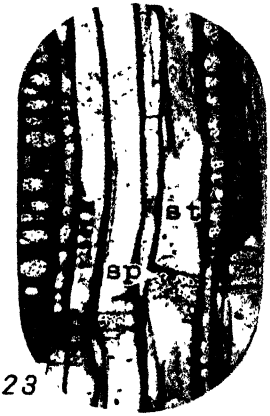
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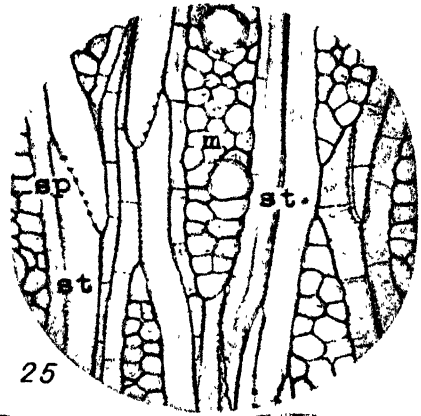
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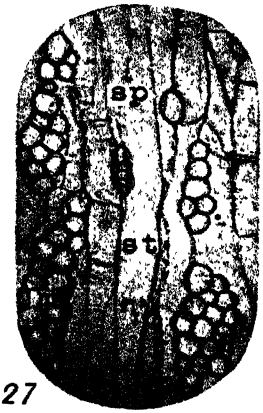
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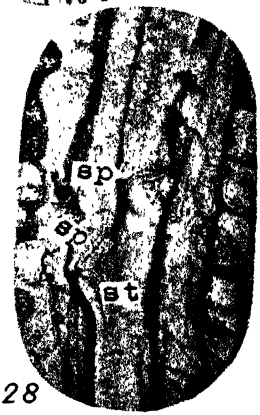
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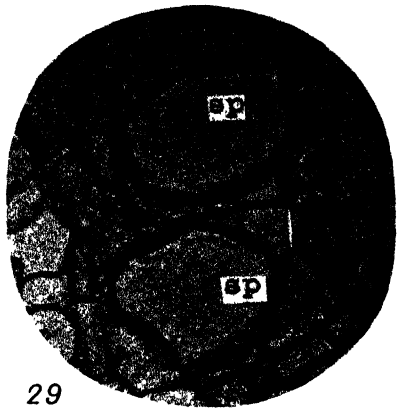
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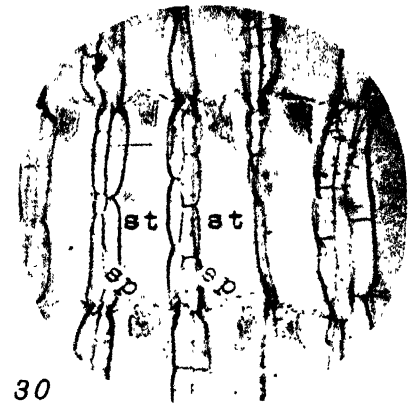
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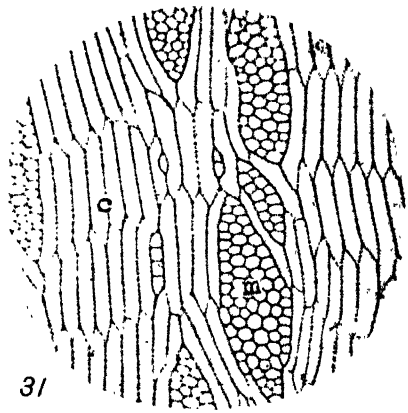
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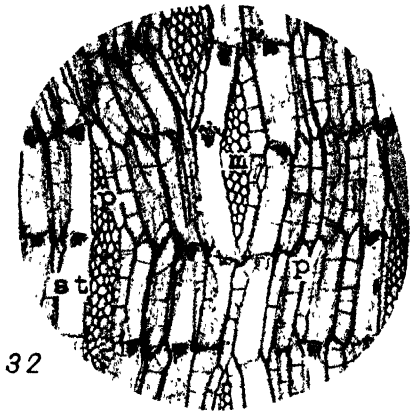
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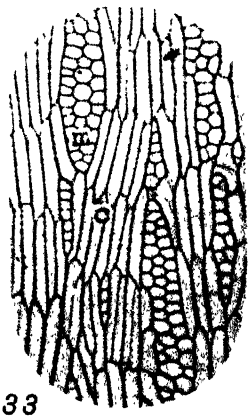
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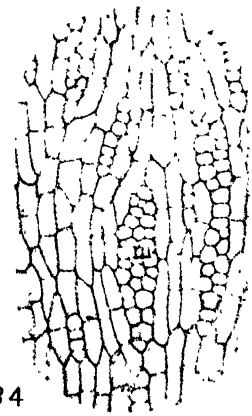
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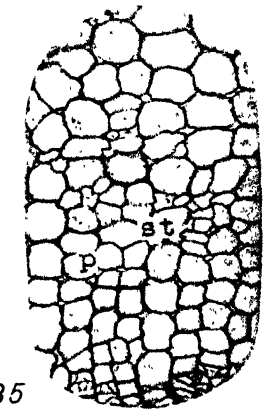
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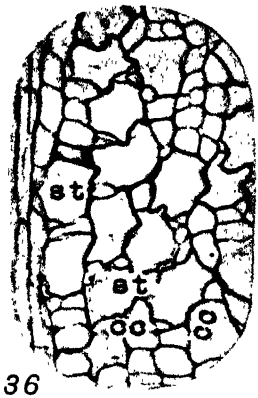
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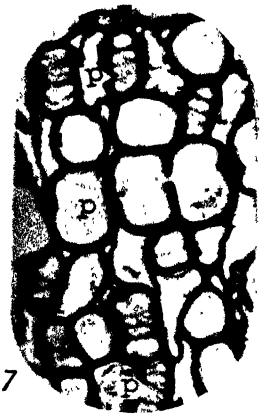
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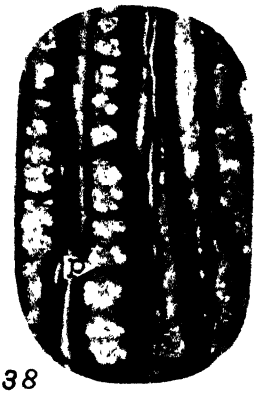
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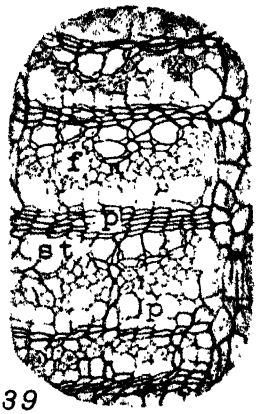
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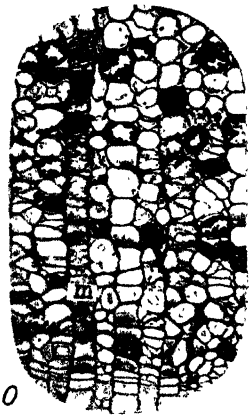
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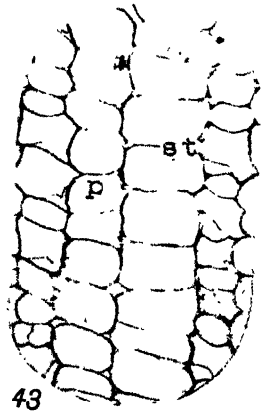
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CELL DIVISION BY FURROWING IN MAGNOLIA

CLIFFORD H. FARR

In "Les centres cinétiques chez les Végétaux" (8), published in 1897, L. Guignard supported his previous contentions as to the existence of centrosomes in Angiosperms by studies on the reduction divisions of certain Dicotyledons, namely: Nymphaea, Nuphar, Limodorum, and Magnolia. Although most cytologists are unwilling to admit that Guignard succeeded in establishing his thesis, yet this must be recognized as a very critical piece of work, involving nearly one hundred careful drawings which bespeak excellent fixation. His figures represent stages in the division of the nuclei, and in addition some of those of Magnolia give stages in cytokinesis. There are included in the text a few paragraphs on cell division proper, but most emphasis naturally is placed on nuclear phenomena. In some of the figures there is shown an equatorial thickening of a few spindle fibers following the heterotypic karyokinesis, but other fibers seem to be uniform throughout. No reference is made in the discussion to the presence or absence of a cell plate. At a little later stage the fibers of the central spindle have a twisted, crinkled appearance in the equatorial region for about one third of their length. Meanwhile an equatorial furrow has developed for a short distance centripetally. It is shown in all stages of interkinesis to be of about uniform diameter at its base and rather sharp at the apex. Just before the nucleoli disappear in the beginning of the homoeotypic division the furrow reaches a depth about equal to the remaining isthmus, and it is said to remain arrested there throughout this mitosis. However, Guignard's figure 25 shows a middle anaphase with the furrow only one half as deep as stated. No figures are shown of the completion of the furrow nor of cytokinesis of any kind after the homoetoypic karyokinesis. It has thus not been fully established that this furrow is related to cell division, nor that there is no cell plate involved in the division of these cells. Guignard's figures represent the mother-cell wall as thickened to about the same extent as is shown by my drawings, although in the former it is of more nearly uniform thickness throughout. However,

in general the drawings of Guignard are in harmony with my own observations recorded below. There are nevertheless certain features in Guignard's work that require further consideration. One concerns the occurrence of a cell plate in the meiotic divisions of Magnolia, another the hour-glass spindle which is shown in his figure 21. In appearance the latter resembles somewhat an oblique section through a cell after the homoeotypic nuclear division is finished, showing two non-sister nuclei and the spindles between them which traverse the heterotypic equator. Since the paper by Guignard, two more publications upon the cytology of Magnolia have appeared. But Andrews (1) and Maneval (13) respectively contribute no additional points on these problems.

A recent paper (6) by the writer purported to establish quadripartition of the pollen mother cells of certain Dicotyledons by a process of furrowing rather than by the typical method of cell plate formation. Since the time of Strasburger's monumental work (15) in 1875, the division of all cells of the higher plants had been supposed to be by cell plates. A search through the literature, however, revealed a number of drawings which unwittingly on the part of their authors point to another interpretation. Furthermore, a study of six genera representing the Compositae, Primulaceae, Solanaceae, and Tropaeolaceae respectively, the last being the one in which Strasburger himself studied the pollen mother cells in this regard, convinced the writer that cell plates are not formed during the division of these cells. Nicotiana was the form most carefully investigated, and it was established that no cell division normally occurs between the first and second nuclear divisions, and that cytokinesis is accomplished by furrowing after the homoeotypic karyokinesis. At this time the four nuclei are tetrahedrally arranged in the cell and a spindle connects each pair of them. A furrow is formed along the equator of each of these six spindles. There are thus four points on the plasma membrane at each of which three furrows meet. At these points the depression of the plasma membrane toward the center of the tetranucleate cell is greatest. These four projections finally become united at the center of the cell, which thus becomes transformed into a four-lobed structure. The isthmuses connecting these lobes gradually become narrower until the division is complete, each lobe becoming one of the microspores. During this process the mother-cell wall swells and at all times fills the furrow, so that a layer of it lies between the microspores as soon

as they are formed. During this division of the cell there is no indication whatsoever of an equatorial differentiation in the spindle, nor does such occur immediately after the heterotypic nuclear division.

There is considerable resemblance between this particular mode of cytokinesis and the typical division of animal cells, except that it is quadripartition and not bipartition. There are also no centrosomes in these plant cells, unless the work of Guignard can be taken as conclusive. The conditions under which these pollen mother cells are formed are not unlike those of such animal cells as eggs, etc., but are quite different from those of most cells of higher plants. These cells are free-floating in the liquid of the anther instead of being in a compact tissue. Beer (3) considers that the walls of these mother cells are composed of pectose, which swells in water, becoming soft and gelatinous instead of being relatively rigid and non-elastic as are cellulose walls. Finally they assume a spherical shape instead of being pressed into parallelopipeds and other flat-faced forms by mutual pressure, as occurs in root tips and other parts of higher plants. This similarity in the conditions which surround the pollen mother cells and many animal cells led the writer to conclude that these conditions have some physicochemical effect in determining the type of cell division and that they might explain the resemblance of the pollen mother cells to the animal cells in this regard and their departure from the mode of cell division characteristic of the cells of most higher plants.

Material and Methods

The present study is based upon cultivated varieties of Magnolia growing at Cinchona Station on the island of Jamaica. Acknowledgments are due Columbia University for the William Bayard Cutting Traveling Fellowship, which made possible the collection of this material. The writer desires also to express his appreciation to Professor R. A. Harper, who offered many helpful suggestions during the prosecution of this work.

The methods employed are the same as were used in the writer's previous investigation (6). Flemming's strong chromic-acetic-osmic solution was used in fixation and his safranin-gentian violet-orange G combination was the stain employed. Living cells were also studied, especially those of *Magnolia tripetala.*

Living Cells

A study of the pollen mother cells of *Magnolia tripetala* L. was begun on April 21, 1917, and continued over the first of May upon material collected in the New York Botanical Garden. Before the initial date the buds and bud scales remained intact, and the buds were not enlarged much over their winter condition. As synapsis takes place, however, there is a very rapid increase in size, and the outer bud scales tear loose from their basilar attachment but remain coherent at their apices forming a sort of "calyptra" over the swelling bud. Not all the buds of the tree develop simultaneously. The external aspect of the bud in the above-noted particulars seems to indicate rather characteristically the exact cytological stage of the mother cells within. Just the relation of the swelling of the bud to the stages of reduction is not exactly clear. The anther swells more rapidly than do the pollen mother cells, so that the latter become suspended in the liquid of the pollen sac.

In living material the spindle fibers are easily discernible in the equators of the cells in the heterotypic division and also between the sister nuclei of the second mitosis. The fibers extending from the furrows to the nuclei are especially prominent. A few cases were noted in which an equatorial streak resembling a cell plate was apparent in the mother cell. In only one case was it found in a cell which also appeared to have furrows.

The usual number of microspores within a single mother-cell wall is four; but the occasional occurrence of supernumerary pollen grains is noted here as in several other flowering plants, notably Fuchsia (2) and Hemerocallis (10). One case was noted in Magnolia in which there were seven cells within the mother-cell wall, but none were found with more than this number. In several cases there were four microspores of normal size and one additional small one, or in some instances two such small cells.

Several stamens were studied with a view to determining the various ways in which the four microspores are arranged with respect to each other. Quantitative results were also obtained to determine the percentage of each type of arrangement. In Table I five types of arrangement are recognized. The accompanying drawings show the characteristic appearance of each type as seen through the microscope. Type 1 is the arrangement referred to by Giesenhagen (7)

as *decussate*, resulting from the spindles of the second meiotic division being at right angles to each other. Type 2 is the result of the spindles being parallel and may be called *quadrate*. Type 3 is the consequence of the two spindles being nearly at right angles to each other, and hence it is an approach to the first type. Type 4 is a similar approach to the second; and Type 5 is the characteristic tetrahedral disposition so common in many Dicotyledons. Types 6 and 7 (not represented by drawings) indicate mother-cells with four large cells and one small cell, and three large cells and one small cell respectively. The figures in the perpendicular columns refer to the number of pollen mother cells of single stamens, designated respectively as *A*, *B*, *C*, and *D*.

TABLE I

Arrangement of Microspores

Type	*A*	*B*	*C*	*D*	Total	Percent
1	34	43	35	35	147	51.2
2	20	15	22	14	71	24.75
3	11	8	13	5	37	12.9
4	4	6	8	5	23	8
5	0	0	0	0	0	0
6	1	3	0	4	8	2.8
7	0	1	0	0	1	.35
	70	76	78	63	287	100.00

These figures show that the tetrahedral arrangement of the microspores is exceedingly rare in Magnolia, if, in fact, it ever occurs at all. It also appears that the percentage of the various types of arrangement is rather markedly constant among various stamens, none of those studied departing very far from the mean. Approximately one half of the total number of cells in each stamen have the decussate arrangement, about one fourth have the quadrate, and deviations from these two types make up the remaining one fourth. In the study of the fixed material some data have been accumulated as to the relative positions of the two spindles during the homoeotypic karyokinesis. In 25 of 45 mother cells the spindles were nearly at right angles to each other. In the other 20 they were nearly parallel. Inasmuch as the latter arrangement is associated with the quadrate disposition of the microspores, it is interesting to note that in both cases these are in the minority respectively. This agreement between the arrangement of the microspores and the corresponding position of the spindles con-

firms the general opinion that the plane of cell division is determined by the direction of the spindle (Giesenhagen, 7), or, perhaps, that they are consequences of the same factors.

Regarding the formation of the furrow, the observations on living cells confirm the statement of Guignard (8) that an incipient furrow is developed during the first division, which is, however, arrested and only completed after the homoeotypic karyokinesis has taken place. In some cases the heterotypic furrow seems to be preceded by an equatorial differentiation, but this always disappears before cytokinesis is effected. During the homoeotypic nuclear division the heterotypic furrow remains arrested, but after the four nuclei are organized this furrow completes the division of the cell. At the same time the homoeotypic furrows are developed across the equators of the homoeotypic spindles at right angles to the heterotypic furrow, resulting in the mother cell's becoming subdivided into four microspores (fig. 10). Whether the heterotypic furrow completes the division of the mother cell into two parts before the homoeotypic furrow is complete is not easy to determine from the living cells, and consequently this question will be left until the study of the prepared slides is discussed.

There are certain terms that are necessary in referring to various parts of the mother cell during the reduction divisions; and it is desirable that the meaning of these words be clearly understood. The furrow which begins during the heterotypic division and is completed after the second mitosis is called the *heterotypic furrow*, even though the later stages of its development follow the homoeotypic karyokinesis. The spindle between the two daughter nuclei of the first division is called the *primary heterotypic spindle*; and the area which it crosses is the *heterotypic equator*. The spindle which crosses this equator after the second nuclear division is known as the *secondary heterotypic spindle*. The spindles connecting the sister nuclei of the second division are the *homoeotypic spindles*, and their equators are crossed by the *homoeotypic furrows*. The region of cytoplasm on the opposite side of each nucleus from the central spindle is the *polar region*, and that beside the nuclei and the spindles is the *lateral region*.

Nuclear and Cytoplasmic Phenomena

The stamen enlarges prior to the initiation of the reduction divisions, so that the pollen mother cells are free within the pollen sacs before

the leptoneme passes over into the pachyneme stage. In some cases the distance between the mother cells is once or twice their diameter, the individual cells being untouched by others on any side (fig. 12). In other instances they are found crowded together, especially in the ends or corners of the pollen sacs, so that each one touches other mother cells to the number of three to five (fig. 2). Even here the intercellular spaces are large (fig. 5), and the cells assume a spherical form. Relatively few cases are to be found in which a deviation from this shape seems to be due to crowding. Some cells in synapsis assume an elongated oval form, and occasionally the nucleus is found at one end of such a cell and the chromatin at the corresponding end of the nucleus. It is as if the chromatin thread had been attracted by something outside the cell and had moved in that direction, causing the nucleus also to migrate toward one end of the cell. Similar abnormal conditions are in evidence in the mitotic stages of these cells. It may very well be that these are wound effects, due to cutting the stamens from the flower before fixing them. Miehe (14) and Christman (4) found similar phenomena, such as the migration of the chromatin and nuclei from one cell into another, and suggested that they might be of a traumatic nature. R. S. Lillie (12) in 1903 found that free nuclei and those of sperms migrate toward the anode, but that cells with a large amount of cytoplasm go toward the cathode. Hardy (9) has more recently (1913) carried on further experiments on the effect of the electric current on cells, in general substantiating the conclusions of Lillie. It may be that these nuclear migrations, as the writer (6) has suggested is the normal procedure in Nicotiana, may be attributable to a disturbance in the electrostatic equilibrium of the cells of the tissue.

All of the pollen grains in a given anther are not in the same stage of the reduction divisions, which is a very helpful fact in the study of pollen formation. There are frequently found lying side by side cells in diakinesis and in the anaphases of the homoeotypic mitosis respectively. It is not, however, usual to find presynaptic stages and young microspores in the same pollen sac.

The stages of transformation within the nuclei are very marked and sharply defined in this material. There is an abundance of the bouquet, leptoneme, pachyneme (fig. 1), diakinesis, and the other phases of the meiotic divisions. No good contraction stages, however, are to be found, excepting the apparently traumatic effects referred to above. The description of the processes involved in the reduction of

the number of chromosomes in Magnolia is not given in this paper, but the series of stages are incidentally employed in determining the sequence of cytoplasmic phenomena. It is scarcely possible to doubt the validity of such stages as the formation of the dispireme (figs. 3 and 20), its transformation into the prochromosomes (figs. 3–5, 20–22), etc. The two series of nuclear and cytoplasmic changes seem to be remarkably constant in paralleling each other. Only during the interkinesis are any cases of possible lack of correspondence found. The photomicrographs and drawings given herewith show very clearly the simultaneous nature of these phenomena.

The number of chromosomes can be determined most easily by a study of the polar view of the metaphase of the homoeotypic division (fig. 9). The following are the results of counts made on a few cells: 45, 46, 45, 43. A fifth cell appeared to have more than any of these, but this was probably due to confusion of the two parts of the same chromosome and to difficulty in determining in each case whether they were pairs or only single chromosomes. The gametophytic number of chromosomes thus seems to be about 45.

The stages up to the telophase of the first meiotic division were described by Guignard (8) and will not be further discussed here. After the anaphases of the heterotypic karyokinesis the chromosomes assemble at the poles in the usual fashion and a nuclear membrane is formed, organizing a nucleus which is flat and discoid (figs. 2 and 26). The spireme is next formed from the chromosomes (figs. 3 and 20). At this time a streak appears midway between the two nuclei in exactly the position in which a cell plate might be expected. This streak takes the orange stain in Flemming's triple combination. Timberlake (16) in his study of cell division in the root tips of onion and the pollen mother cells of larch showed that such an "orange zone," as he called it, precedes the formation of the cell plate, and interpreted it as a preliminary step in cell-plate formation. In Magnolia it seems that the orange zone appears, but in no case is there any evidence of its being followed by the formation of a cell plate. It must be that the conditions for cell-plate formation obtain at first, but that they do not continue to exist, or that some factor enters in to interrupt the process. I was able to find this orange zone in only five or six out of fifty or more cells of Magnolia which, judging from the nuclear phenomena, were in exactly the same stage. It is thus by no means certain that it is formed in the division of every mother cell. Neither Andrews (1),

Maneval (13), nor Guignard (8) suggests even the possibility of the presence of such a structure. That it is in some instances formed cannot be doubted, as is shown by the photomicrograph (fig. 3) and the drawing (fig. 20) below. It is never seen to extend the entire distance across the mother cell, but in one or two instances the nuclei were closer to one side than to the other, and the orange zone reached the membrane on that side; in a few cases it was found to be interrupted, though this may have been caused by the shock of fixation. Cells in later stages can all be easily detected by the fact that the nuclei at the time of the presence of the orange zone are very flat and near together; whereas upon the disappearance of this body the nuclei round up and separate. Juel shows incomplete cell plates in Hemerocallis (10) and Carex (11), in the latter of which the cell plates are ephemeral. The case of Magnolia is slightly different, for it is not the cell plate which is ephemeral here, but rather the orange zone. Furthermore, in Magnolia the disappearance of this equatorial structure is followed by furrowing, which is not the case in the forms studied by Juel.

After the disappearance of the orange zone the nuclei enlarge and in them the nucleoli appear (figs. 4 and 21). The latter are at first small and there may be one or more of them in each nucleus, but they usually enlarge up to the time of the second division, keeping pace with the enlargement of the nucleus and even exceeding the latter in some instances. In fact, a nucleolus may become so large at times that it extends from the polar to the equatorial side of the nucleus (fig. 8). All except the large nucleoli are perfectly spherical. The latter often appear bell-shaped, like the starch grains of ginger. In addition to one or two large nucleoli in each nucleus at this stage there are also some small ones. A surprisingly large number of these cells in interkinesis have the large nucleoli exactly opposite each other in the nuclei of the same mother cell (figs. 6 and 21), though they may be in the center or at either side. Large nucleoli were found opposite each other in 59 cells, while in only 5 were they otherwise arranged.

While the nucleoli are enlarging, the furrow makes its appearance. It is difficult to arrange a series of stages in nuclear changes and furrow formation during interkinesis. However, the initiation of furrow formation is reasonably well indicated by nuclear phenomena. After the orange zone has disappeared the nuclei slowly enlarge and pull apart (fig. 5), becoming more nearly spherical (fig. 6). Before they attain the maximum distance apart the spireme has become completely

transformed into the prochromosomes of the resting nucleus. After nuclear migration has ceased and the nuclei have reached their maximum size, they again become much flattened at right angles to the direction of the heterotypic spindle (figs. 7 and 22). This is unquestionably in preparation for the homoeotypic karyokinesis (fig. 8). The breaking down of the nuclear membrane takes place before the disappearance of the nucleoli, and a multipolar spindle is organized about the chromosomes. These latter appear to arise directly by the enlargement of the prochromosomes and not by the interpolation of a spireme stage. Hence the prophase of the homoeotypic division is not exactly the reverse of the telophase of the heterotypic. As the multipolar stages progress the nucleoli continue to decrease in size, until by the time the bipolar spindles are formed they have completely disappeared. During the homoeotypic karyokinesis there is usually present an area of orange-staining homogeneous material on that side of each spindle that is toward the equatorial region of the previous division. It is often about equal to or wider than the spindle itself and extends for varying distances around to the polar side, but is always thicker toward the equator. In it no fibers are to be seen and the fibers in the region between the two orange areas become fewer and fewer as the prophases and metaphases progress. It is not improbable that the substance which composed the fibers of the primary heterotypic spindle becomes dispersed into the homogeneous orange-staining material. Only in rare instances is a fiber found crossing the heterotypic equator during the anaphases (fig. 9).

After the chromosomes are assembled at the poles in the telophases of the heterotypic karyokinesis, a nuclear membrane is formed in the usual fashion. No spindle fibers were found to be organized across the homoeotypic equator until this membrane appears (fig. 23). As the fibers make their appearance across the heterotypic equator the orange area above mentioned slowly vanishes. No orange streak or other equatorial differentiation is found in any of the spindles of the second mitosis. In fact, no cytoplasmic changes at all are distinguishable until the nuclei have enlarged (figs. 11 and 12) and the dispireme has gone over into the prochromosome stage. The furrows develop at this time (fig. 24). The details of this process will be discussed below.

The thickening of the wall is not as extreme as in Nicotiana (6), but is nevertheless very evident. It has the same staining reaction and homogeneous appearance as in the species of Dicotyledons for-

merly studied by the writer. In Magnolia, however, the thickening takes place more nearly uniformly over the entire surface of the cell, especially where the wall is not in contact with the walls of other cells (fig. 11). The writer does not find as great a degree of uniformity in the thickness of the cell wall as the figures of Guignard (8) indicate. It is ordinarily about one twenty-fifth of the diameter of the mother cell during the heterotypic division, and in interkinesis it thickens slightly, so that it becomes about one sixteenth to one twentieth of the diameter (figs. 13 and 23). During interkinesis the mother cells are in many cases elongated in the direction of the axis of the heterotypic spindle.

The Furrowing Process

In Nicotiana (6) there is neither a cell plate nor a furrow formed between the first and second nuclear divisions. In Magnolia, however, the formation of both of these structures is initiated, but neither is brought to completion before the homoeotypic karyokinesis. The former completely disappears, whereas the latter is arrested (figs. 6 and 8). Guignard's paper indicates that this furrow remains where it is arrested during the entire second division; but I am of the opinion (fig. 9) that, occasionally at least, it may recede somewhat. The furrow appears in the plasma membrane at the equator of the heterotypic spindle at a considerable interval of time after the stage of the ephemeral orange zone. Meanwhile the nuclei have enlarged and separated considerably (figs. 5 and 22), and the nucleoli have been developed. The nuclei remain in almost this same condition until they flatten in preparation for the next division. During this period of resting of the nucleus the furrow is being formed. It is obvious that there is some difficulty in arranging a series of stages in furrow formation on the basis of nuclear changes when there is no such series of collateral processes with which to compare it. One finds a great variety of furrows; some appear in section as minute mucronations, others are broad invaginations, some have sharp edges, others are rounded; some are shallow, others are deep. Any arrangement of these in a series must be merely hypothetical, but an attempt at seriation is not without value. It may be supposed that the furrow begins as a sharp, knife-like edge which deepens gradually and broadens at the base. The sharpness may be retained until the furrow reaches the maximum depth attained before the homoeotypic mitosis. There-

upon it probably becomes rounded at the end, of a more nearly uniform width, and finally may recede somewhat. In case it recedes, it doubtless becomes broadened to a greater extent (fig. 9). In some mother cells in the homoeotypic karyokinesis there appears to be very little if any furrow at all, whereas very few cells are to be found in which two fully developed nuclei occur without some sort of a furrow being present. All of which is evidence that a furrow is always formed during interkinesis and then may recede even to obliteration.

The cell-wall material always fills the furrow and appears as a jelly-like mass. The second division furrow is always narrow in section (figs. 13 to 18), and never broadens at the base like that of the first division. It is thus reasonable to assume that when the furrows begin to develop after the homoeotypic nuclear division, they progress continuously until the cell is divided, in this way differing from the first furrow which is known to be arrested in its development for a time. This sharp-edged furrow is the only kind that is to be found after the second division, and it is entirely probable that the heterotypic furrow begins in this way, as suggested above. If we think of the plasma membrane as the active agent in furrowing, and the attraction between it and the nuclear membranes as the force involved, this tension would be destroyed upon the disintegration of the nuclear membranes in the prophases of the homoeotypic karyokinesis, and the cell turgor would express itself in pressure against the plasma membrane, causing the equatorial furrow to recede or at least to flatten out somewhat. Conklin (5) and others have shown that in certain animal cells the cleavage furrows are arrested as a result of the cells being placed in hypertonic solutions, while the division of the nuclei and centrosomes may continue.

After the homoeotypic nuclear division, the daughter nuclei are at first narrow in section and close together (fig. 23), just as is the case after the first division. As the spireme passes over into the prochromosome stage the sister nuclei enlarge (fig. 12), become more nearly spherical (fig. 11), and pull apart slightly, but not so much as in the heterotypic division (fig. 15). No orange zone (fig. 23) or other semblance of an equatorial differentiation in the central spindle is found in the homoeotypic division. As soon as the nuclear membranes are formed, fibers appear across the heterotypic equator and soon definite spindles are organized. Because of the proximity and narrowness of the daughter nuclei, at first these spindles are not completely organized;

but later they are seen to be composed of fibers that run from each of the two sister nuclei on the one side to each of the two sister nuclei on the other. So that there are fibers running between every pair of nuclei in the tetranucleate cell, just as in the tetrahedral pollen mother cells of Nicotiana (6). In the latter case there are thus six distinct spindles, but in Magnolia there is some question as to whether they should be considered as six separate spindles or as two simple spindles and one compound spindle (figs. 14 and 24).

The formation of the spindle fibers in the heterotypic equator after the homoeotypic karyokinesis presents a problem which has as yet never been satisfactorily solved. Most central spindles are organized as a consequence of mitotic karyokinesis, just as occurs in the formation of the primary heterotypic and homoeotypic spindles in these cells. But in multinucleate cells that are formed by successive mitotic division of a primary nucleus, one or more spindles are apparently organized in some other way. In Nicotiana and several other Dicotyledons (6) four spindles are thus formed; in Magnolia there is one compound spindle so organized. In the last-named form this compound spindle lies in exactly the same place as did the primary heterotypic spindle, although the fibers in the two spindles are not arranged in the same way. The primary heterotypic spindle completely disappears as the homoeotypic division begins, and no semblance of it is found until after the new nuclei are formed. In the telophase a large number of fibers again appear. Whether these represent a reorganization of the primary heterotypic spindle, or the formation *de novo* of a large number of fibers is not entirely clear. The cells in the metaphase and anaphases show no spindle fibers (fig. 9) across this area. The cytoplasm in the equatorial region is granular, or in some cases apparently alveolar, and stains blue with the Flemming's treatment. The area of this type comprises about one third of the space between the two nuclei while the other two thirds between it and the two nuclei respectively is of a different composition. This latter takes on the orange stain and is homogeneous in appearance rather than granular. This orange area is widest on the equatorial side but extends around toward the opposite side for varying distances until it gradually disappears. No indications of fibers are to be found in this orange area, but it is not unlikely that it may be composed of material which once made up the fibers of the primary heterotypic spindle and which will again form the secondary one. In figures 24 and 25 of the writer's paper on

Nicotiana (6), it was shown that orange-staining granules apparently arrange themselves in rows to form the fibers of the new spindles after the second nuclear division. It is scarcely conceivable that so many fibers could be present in the cytoplasm as such during mitosis and not be detected. A greater probability seems to be that the material of the old spindle may have become dissipated throughout the cytoplasm and have formed the orange areas, later to be used again in forming the new fibers.

The furrows after the second division begin as sharp cutting edges formed by the infolding of the plasma membrane at its juncture with the equator of each spindle respectively (fig. 24). It will be observed that the secondary heterotypic spindle meets the plasma membrane at the summit of the heterotypic furrow, so that a new furrow is superimposed on the arrested one. The new one is always narrower than the older (figs. 14 and 24), so that their place of union remains marked throughout the process of furrowing. This superposition of the secondary heterotypic furrow on the primary one, as well as the usual elongation of the cell parallel to the heterotypic spindle, results in the equator of the latter being very narrow as compared with that traversed by the two homoeotypic spindles (figs. 13 and 25). In fact, the width of the secondary heterotypic spindle is frequently less than that of either homoeotypic spindle alone. Consequently, if all the furrows deepened with the same rapidity the cell would be divided into two parts first and then each of these would undergo bipartition by the homoeotypic furrows. This seems to be only rarely the case, and it is probable that the heterotypic furrow always develops somewhat more slowly than the homoeotypic furrows (fig. 15). In most instances there is apparently perfect quadripartition (fig. 17) due to the retardation of the speed of development of the secondary heterotypic furrow; but in a few cases (fig. 18) it is probable that the latter is completed slightly before the homoeotypic furrows have entirely closed their isthmuses.

Cases of normal cell plate formation are quite abundant in the cells composing the anther walls of these same stamens (fig. 19). It may thus be concluded that all cells of Magnolia except the pollen mother cells divide in this way. The plate appears in the center of the spindle first, as described by Strasburger (15) and Timberlake (16), and then develops centrifugally in the equator accompanied by the formation of new fibers and the widening of the spindle. This process continues

until the cell is completely partitioned, and the cell plate gives rise to plasma membranes between which the cell wall is formed. No indications of furrows or similar structures are discernible in these cells. It is observed that this cell plate formation occurs simultaneously with the organization of nuclear membranes and the union of the chromosomes to form the spireme. It is practically finished before the nuclei begin to enlarge and separate. The furrowing in both the heterotypic and homoeotypic divisions, on the other hand, is subsequent to and not simultaneous with these nuclear changes. It thus appears that the furrow and the cell plate are not homologous structures though they accomplish the same end. The formation of a furrow in the pollen mother cells must thus be regarded as taking place only when no cell plate has been previously formed. In other words, if a cell plate is for any reason not formed there is a furrow developed at a later time. The conditions incident to the two processes may not be at all the same. It is entirely likely that the conditions for furrowing exist in all cells of both plants and animals, but in plants they can not usually express themselves on account of the previous cell plate formation. Thus animal and plant cells may be potentially identical as to furrowing, but most plant cells in addition have the power of cell plate formation. The writer has previously suggested (6) that the latter process is related to the presence of a cellulose cell wall and the inability of the cell to enlarge in response to osmotic pressure, and hence is characteristic of plant cells alone.

The cause of the heterotypic furrow's remaining arrested until after the second nuclear division is a matter of considerable interest. The suggestion has been made by several writers that the lack of cytokinesis between the first and second karyokineses of the pollen mother cells of many plants is due to too short a period of interkinesis. The observations of the writer on Magnolia indicate that the period of interkinesis in this form is by no means short as compared with the time required for mitosis. The greater number of cells in my preparations were in interkinesis rather than in either the heterotypic or homoeotypic mitoses. If, as the writer has previously suggested (6), the furrowing is the consequence of a mutual attraction between the nuclear membranes and the plasma membrane, then an arrest of the furrow would be due either to a change in this force of attraction, or to the fact that the nuclei have such a size and position as to cause the resultant of the attracting forces to become zero after a certain

depth has been reached. Thus far no instance of bipartition by furrowing has been established in the higher plants, such as is so common in the animal kingdom. In the latter it is evident that the centrosomes are much smaller and much farther apart than are the nuclei in these mother cells, and hence if these centrosomes be the attraction centers, a resultant of forces might lead to complete bipartition. If, however, in these pollen mother cells the nuclei are the attraction centers, it may be that they are too large and too close together to accomplish complete partition in a binucleate cell. That the nuclear membranes are probably important in furrowing is indicated by the fact that furrowing is not resumed until the nuclear membranes are reformed in the telophases of the homoeotypic mitosis.

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EXPLANATION OF PLATES XXX–XXXI

Plates XXX and XXXI are photomicrographs of cells of Magnolia, all being pollen mother cells except figure 19. The magnification is 650, except in figures 10 and 19.

Plate XXXII presents drawings made with a Spencer microscope with tube length 17.5 cm., objective 1.5 mm. N. A. 1.30, and ocular 6 ×. Magnification 900

PLATE XXX

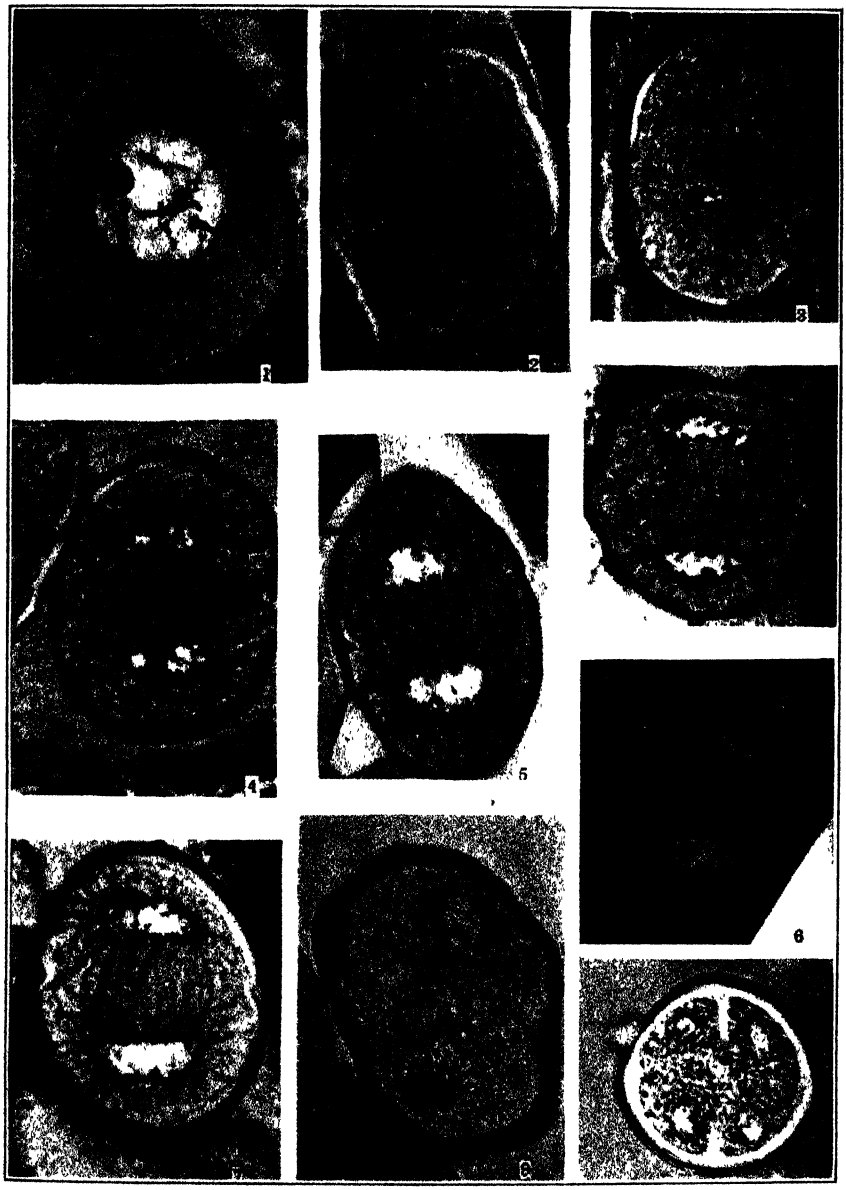
FIG. 1. Pachyneme of prophase of heterotypic mitosis.
FIG. 2. Early telophase.
FIG. 3. Nuclear membrane and orange zone forming.
FIG. 4. Nuclei larger, orange zone disappeared.
FIG. 5. Furrow beginning.
FIG. 6. Furrow well formed, nuclei in resting condition.
FIG. 7. Nuclei broader and larger.
FIG. 8. Nuclei very broad, nucleolus large.
FIG. 9. Anaphase of homoeotypic mitosis.
FIG. 10. Living cell showing furrows.

PLATE XXXI

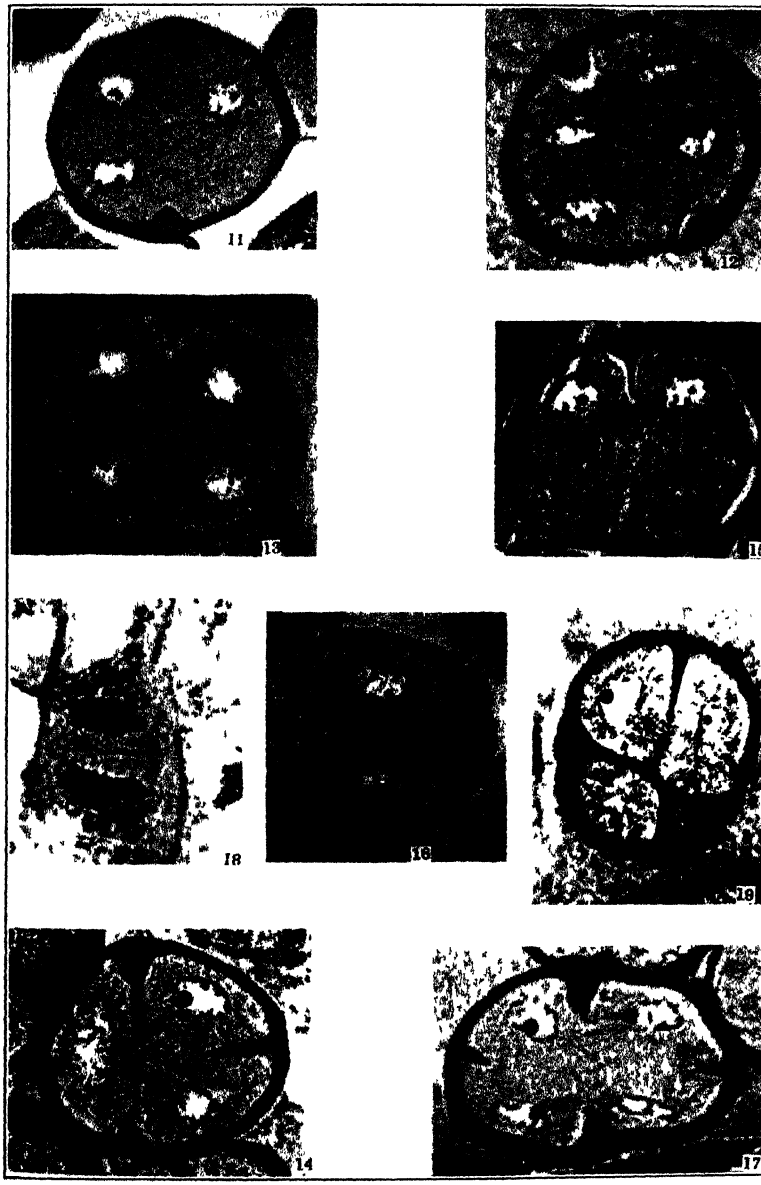
FIG. 11. Nuclei reorganized after second division.
FIG. 12. Same as Fig. 11, with spindles parallel.
FIG. 13. Same stage with cell spherical.
FIG. 14. Homoeotypic furrows forming.
FIG. 15. Furrow deep across heterotypic equator.
FIG. 16. Polar view of same stage as Fig. 13.
FIG. 17. Late stage in quadripartition.
FIG. 18. The completion of the furrow.
FIG. 19. Cell plate formation in a cell of the anther wall.

PLATE XXXII

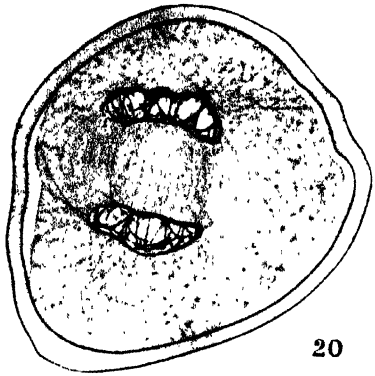
FIG. 20. Dispireme stage following the heterotypic karyokinesis.
FIG. 21. After the orange zone has disappeared and before the furrow is formed.
FIG. 22. Furrow formation.
FIG. 23. Following the homoeotypic karyokinesis, no orange zone.
FIG. 24. Furrow being resumed.
FIG. 25. Furrows partially completed.



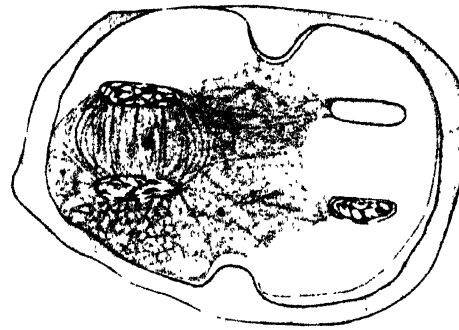
FARR: CELL DIVISION IN MAGNOLIA.



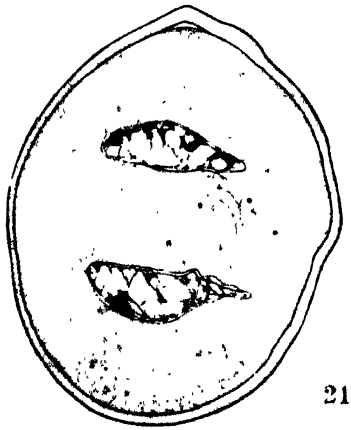
FARR: CELL DIVISION IN MAGNOLIA.



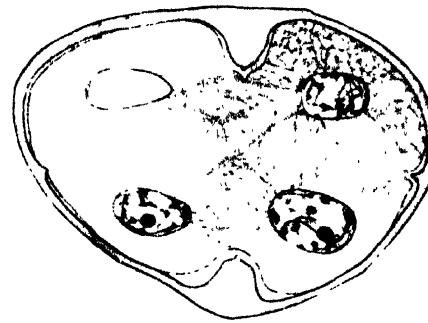
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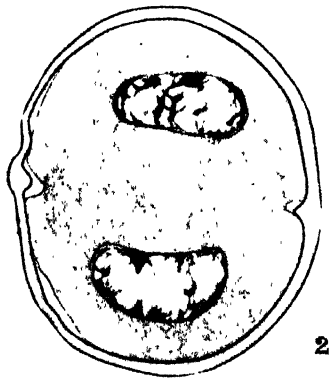
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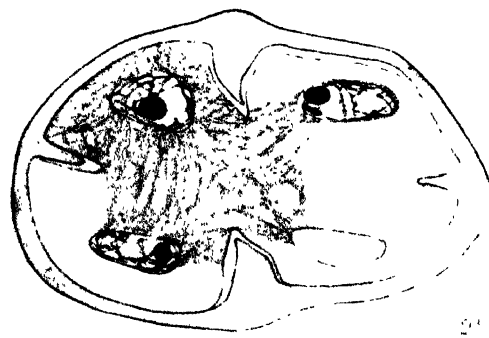
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THE CYTOLOGY OF EOCRONARTIUM MUSCICOLA

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The view of Brefeld (5) that the promycelium of the Uredinales and Ustilaginales is homologous with the transversely septate basidium of the Auriculariales has received general acceptance. His theory, based on a morphological study of the two structures, has been substantiated by the results of subsequent cytological investigations on members of these three groups and of the higher Basidiomycetes. Consequently the Auriculariales are regarded as closely related to the Uredinales, and as probably intermediate in origin between them and the higher Hymenomycetes. Comparatively little is known, however, concerning the nuclear history and general cytology of members of this order. Only species of the genus Auricularia have been examined, and here the facts are only partially determined. The investigation of members of other genera is therefore desirable, and should shed further light on the phylogeny of the Basidiomycetes.

A preliminary examination of stained sections of the sporophore of *Eocronartium muscicola* (Fries) Fitzpatrick, made in connection with the writer's (12) study of the parasitism of this species, disclosed the fact that unusually large nuclei make this form a favorable subject for cytological investigation. The present paper is an outgrowth of this discovery, and embodies the results of an investigation of the cytology of this species conducted during the past three years.

MATERIALS AND METHODS

Eocronartium muscicola is an obligate parasite occurring on a considerable number of mosses of various genera. It produces small Typhula-like sporophores at the apices of the branches of the moss gametophore, in which it exists as a perennial, intracellular parasite.

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A detailed account of its parasitism, life history, and morphology has already been given in the writer's previous paper (12), and the facts need not be repeated here.

The present investigation of the cytology of the species is based on material taken from a single host, *Climacium americanum* Brid., all the collections having been made by the writer in the vicinity of Ithaca, N. Y. *Climacium americanum* is a large moss, and the fungous sporophores produced on its branches are larger than those developed on smaller species. They are consequently more favorable for study. The large size of the host, moreover, renders it especially suitable in connection with the staining of the endophytic mycelium. Fortunately the fungus has been collected more abundantly on this host than on any other.

Under favorable weather conditions the young sporophores, which make their appearance at the tips of the gametophoric branches, undergo rapid elongation, and within a period of two weeks begin to bear basidia and form spores. If dry weather prevails their development is retarded, and material favorable for cytological examination is not easily procured in the field. Parasitized host plants were, therefore, collected in the early summer, and placed in the greenhouse under conditions favorable for growth. Abundant moisture was provided and normal fungous sporophores developed in great numbers. In this manner excellent material illustrating all stages in the development of the fruit-body and its hymenium was easily obtained.

Sporophores intended for subsequent cytological study were removed from the host at varying intervals and given a preliminary microscopic examination before they were placed in the fixing solution. A brief examination of the individual sporophores under the lower powers of the microscope proves of considerable service in demonstrating the presence of spores. Their comparative abundance furnishes a criterion for the determination of the age of the basidia. Since basidia of practically all stages of development are commonly found together on a single sporophore, this preliminary examination gives, however, only an indication of the predominating stages present in the hymenium. Sporophores collected in the field at different times during the growing season were placed in the fixing solution and subsequently studied in comparison with those developed in the greenhouse.

For study of the endophytic mycelium, diseased host plants were

collected at all seasons of the year. Portions of the main axes of the gametophores and their branches as well as pieces of the procumbent "stolon" were removed from the plant and placed in fixing solution. Material was selected from the newer light green branches and from the dark green parts developed the previous year. A careful examination of an infected individual shows that the hyphae are present in both the new and old growths. Healthy host plants were also collected, and portions of these were used for comparison.

The most satisfactory fixing agent used was the medium strength solution of chromo-acetic acid recommended by Chamberlain (6). It gave wholly satisfactory fixation, while solutions containing osmic acid proved less useful. Air was removed from the material by means of a suction pump. This is particularly necessary when pieces of the host plant are treated, since the branches of the gametophore are sheathed by small imbricated leaves, and the stolons are covered with numerous closely matted rhizoids. The majority of the sections were cut 5μ in thickness; none exceeded 7μ. They were for the most part cut parallel to the long axis of the sporophore or gametophoric branch, few transverse sections being made.

Various combinations of stains were used. Heidenhain's iron alum-haematoxylin was given a thorough trial but proved unsatisfactory. It was used alone and in combination with certain other stains such as Congo red, fuchsin, and erythrosin. Small cytoplasmic granules stain deeply with haematoxylin, and obscure the details of the nuclear structure. The triple stain of Flemming gave better results. After a few trials it became evident that in the study of *Eocronartium muscicola* the use of the shortened method recommended by Harper and others is far superior to the longer schedules previously employed. A sharper differentiation of the chromatin and achromatic structures, fibers, centrosomes, and nucleoli is obtained. This fact combined with the economy of time resulting from its use makes the shorter method much more advantageous. The three stains in the combination were applied for various periods of time, the following schedule proving most satisfactory: safranin 1 minute, gentian violet 5–15 minutes, orange G 10–20 seconds.

The nuclei in the various structures of the fungus seem to possess an almost equal affinity for the stains, while the cytoplasm in the basidia, spores, and hyphae is affected relatively little. The cytoplasm of the host cells stains slowly and does not obscure the hyphae appreciably, but the host nuclei stain sharply and rapidly.

The spores of this fungus can be induced to germinate very readily in tap water or synthetic solutions practically 100 percent germination being obtained in twenty-four hours. The germinated spores were stained and permanent mounts of these made, the following method having been used.

A drop of sterilized water was placed in the center of a sterilized glass slide, and a sporophore of the fungus was washed in this. Since the spores fall away easily, this procedure resulted in a spore suspension containing hundreds of spores. After a microscopic examination to determine whether a sufficient number of spores were present, this drop was drawn up into a small pipette. Several clean cover glasses were then provided, and a small drop of this spore suspension was transferred from the pipette to the center of each. These cover glasses were then inverted over van Tieghem cells. The hanging drop culture which was thus obtained could be examined frequently under the microscope, and the germination of the spores watched.

When spore germination had progressed to the desired point the cover glass was carefully removed from the cell, and the spore suspension subjected to the fumes of osmic acid. The stopper was removed from a narrow-mouthed bottle containing a strong solution of the acid, and the cover glass was placed over the opening so that the drop hung inside enveloped in the fumes. Several minutes of this treatment were sufficient to effect fixation. In some cases this drop was then stippled upon a slide smeared with albumin fixative, the method recommended by Harper (17) being followed from this point. In other cases it was allowed to dry on the cover glass. The procedure, recommended by Harper, of diluting the suspension of germinated spores with a drop of Flemming's fixing solution, results in a scattering of the spores and gave less desirable preparations. After the spores had dried down on the cover glass or slide they were carried through the usual staining schedule.

Endophytic Mycelium

The hyphae of *Eocronartium muscicola* in the tissues of the host (figs. 1–4) are more irregular in shape and more frequently branched than those composing the sporophore. They vary considerably also in size, the threads in the larger cells of the basal portions of the gametophore being in general of larger diameter than those in the smaller embryonic cells of the apical point or those in the cells of the leaves.

Great variation is shown also in the length of the individual cells of the mycelium, some cells being many times longer than others (figs. 1, 2). The transverse septa are sharply defined. In stained material they cannot be easily overlooked. At or near the center of each septum there is usually present on one or both surfaces a large, hemispherical or disc-like, deep-staining body (figs. 1–4). These structures also occur on the septa of the hyphae making up the sporophore (figs. 5, 6, 14, 16). They stain deeply with gentian violet and Heidenhain's iron alum-haematoxylin. It is interesting to note that Levine (32), who found what appear to be identical structures in several species of Boletus, states that they stain a deep red with safranin.

These disc-like structures are described as of common occurrence on the cross walls of hyphae in many Basidiomycetes and certain Ascomycetes, and are frequently figured. They were apparently first mentioned by Hoffmann (22). Strasburger (49) found them in *Agaricus campestris* and states that they mark the position of intercellular pores, thus indicating protoplasmic continuity. They have subsequently been found by Rosenvinge (46) in *Clavaria vermicularis*, by Ruhland (47) in *Lepiota lilacino-granulosa*, by Harper (19) and Nichols (38) in *Coprinus ephemerus*, by Levine (32) in *Polystictus versicolor*, *Polyporus adustus*, *Polyporus betulinus*, *Polyporus destructor*, *Boletus granulatus*, and *Coniophora cerebella*, and by Kniep (27, 28) in *Coprinus nycthemerus*, *Corticium varians*, *Corticium serum*, and *Polyporus destructor*. They have also been described by Harper (18) in *Pyronema confluens*, and by the writer (11) in *Rhizina undulata*. They are apparently common in many of the higher fungi, but in certain species are stated definitely to be absent. Their occurrence is not associated especially with either clamp connections or hyphal anastomoses. The specific function of these bodies is doubtful, and no attempt has been made by the writer to determine their exact nature. The difference between the writer's results and those of Levine with reference to their affinity for stains indicates, however, that their composition is not uniformly the same.

The cells of the endophytic hyphae in *Eocronartium muscicola*, in all the cases observed by the writer, are clearly binucleate (figs. 1–4). A painstaking search through many slides has failed to reveal uninucleate or multinucleate cells. Since the transverse septa in stained material are sharply defined, the number of nuclei in a given cell cannot be easily mistaken. As is to be expected, occasional cells contain-

ing four nuclei are found. The position of the nuclei in the cell in such a case indicates, however, that conjugate division has recently taken place, the intervening septum having not yet formed. The rarity of these four-nucleate cells shows that the septum is laid down rapidly immediately after the completion of the mitosis. The hyphae in all parts of the host from the stolons to the tips of the branches have been given critical examination, and in all the material studied the cells are undoubtedly binucleate throughout. Branches from infected plants have been placed in the fixing solution at all seasons of the year, but no variation from the binucleate condition has been observed. If uninucleate or multinucleate cells occur in the endophytic mycelium, they must be present in early stages immediately following infection. No material showing these early infection stages has been collected. The failure of the writer (12) to obtain infection has, consequently, rendered impossible the determination of the point of origin of the binucleate condition.

The two nuclei in any given cell are of practically the same size. Those in different hyphae, and in different cells of the same hypha, vary considerably in diameter. None of the nuclei in the endophytic hyphae reach the large dimensions of certain of those in the interior of the sporophore. They are, therefore, less favorable for study of conjugate division. In fact, none of the details of mitosis have been clearly observed in the hyphae in the host cells.

The Sporophore

The binucleate condition is maintained in all the cells of the hyphae composing the sporophore (figs. 5–16). Uninucleate or multinucleate cells have never been observed. The nuclear pairs divide in all cases by conjugate division, and the daughter nuclei are soon separated by the formation of transverse septa. The two nuclei of any pair are of practically the same diameter, but great difference in size is evident between nuclei of different cells (figs. 5, 6). The greatest variation also exists in the diameter of the hyphae and in the length of the cells. In general, the hyphae lying near the center of the sporophore are of larger diameter than those near the periphery.

The variation in size of the nuclei in the cells of the sporophore is remarkable. Some of these reach a diameter nearly as great as that of the fusion nucleus of the basidium, while others in adjacent hyphae are more minute than the nuclei of the spores. It is difficult to explain

this great difference, though two factors are evidently involved. The large amount of cytoplasm in the larger cells probably necessitates a corresponding increase in the mass of the nuclei. Also it is evident that the nuclei increase greatly in size as they pass into mitosis, the largest nuclei observed being in process of division.

The two nuclei of a cell, when in the resting condition, usually lie a considerable distance apart, frequently in opposite ends of the cell. This is especially true of extremely long cells. When preparing to divide, the nuclei migrate toward the center of the cell, approach each other, and often come to lie in actual contact. In the majority of cases nuclei at this stage are of so large a diameter that it is impossible for them to pass each other in the thread, or to assume the side-by-side position typical of conjugate division. At all stages the nucleolus is evident as a spherical homogeneous body, staining deeply with safranin, and easily distinguishable from the chromatin material in the nucleus. The chromatin stains sharply, and in stages preceding the formation of the spindles is frequently contracted into a compact mass in one side of the nucleus (figs. 6, 7), the nucleolus occupying the other. In poorly stained preparations a pair of nuclei in this condition have the appearance of four small nuclei. When well stained the nuclear membranes are evident, and the two deep-staining bodies in each nucleus then are seen to lie in a common, hyaline nuclear cavity.

The two nuclei pass from the resting condition into mitosis together. The formation of the spindles is not, however, always exactly simultaneous (figs. 9–14). At all stages up to late anaphase each nucleus possesses a well defined membrane, and the spindle, which is intranuclear, stands out sharply in the nuclear cavity accompanied by the nucleolus. The spindles are not necessarily parallel. Moreover they lie without any reference to the long axis of the cell.

The spindle fibers stain clearly, and there is visible in some cases at each pole of the spindle a more deeply staining point. This is doubtless the centrosome, but its minuteness precludes any study of its structure. In certain cases it has the appearance of a short rod resembling that described and figured by Harper (20) in Phyllactinia. The writer has never seen any indication of astral rays. The preparations examined have proved very favorable for a study of division stages. Equatorial plate stages are especially numerous, and in many of these cases it is possible to count the chromosomes with a reasonable degree of certainty. All the evidence accumulated shows the number

to be four. Occasionally also four chromosome-like bodies can be seen in a nucleus which shows no indication of a spindle, and which is evidently in an early prophase (fig. 8).

When the chromosomes begin to pass toward the poles the nuclear membrane is still evident, but it disappears soon afterward. In figure 15 the membrane is absent, and the chromosomes are shown scattered over the spindle. Here also the chromosome number is clearly shown to be four. The two spindles orient themselves parallel to the long axis of the cell, and soon come to lie side by side in typical conjugate division (fig. 16). The nucleolus is drawn into the spindle and passes toward one of the poles. In late telophase it is incorporated in one of the daughter nuclei.

Although in metaphase and early anaphase the spindles do not occupy the position characteristic of conjugate division, they pass more or less definitely into this position before late telophase, and it is evident that the two daughter nuclei which migrate into each end of the cell are not in any case sisters. At the completion of the division the four resulting nuclei round up and remain for a brief period as well defined nuclei in a single cell (figs. 17, 18). The fact that these four-nucleate cells are rarely found indicates that the transverse septum is formed quickly. After the laying down of the septum the two resulting cells elongate, and the nuclei in each drift apart.

As the sporophore approaches maturity, the hyphae at its periphery undergo a slight amount of branching, the terminal portions turning out at right angles to the long axis of the sporophore. A more or less definite palisade layer is thus formed. The terminal cells later undergo further growth, and develop into basidia. The basidia do not stand close together, and a definite hymenium, such as occurs in most of the higher Basidiomycetes, is not formed. Paraphyses are absent, and no sharp differentiation of the fruit-body into subhymenium and trama occurs. The hyphae composing the sporophore interweave only to a slight degree, and a loose tissue results in which an individual hypha may be traced from the basidium far back into the fruit-body.

Nuclear Phenomena in the Basidium

When the basidium is merely the undifferentiated terminal cell of one of the hyphae composing the sporophore it contains two small nuclei similar to those present in other cells of the thread. As it

enlarges these nuclei increase rapidly in size (figs. 20–23). They also become more sharply staining, and migrate toward the center of the cell. Here they soon come into actual contact and finally fuse (fig. 24), the membranes of the two nuclei being dissolved at the point of contact and a common cavity resulting. The chromatin strands of the two nuclei intermingle completely even before the deep constriction about the common nuclear cavity has disappeared. The resulting fusion nucleus soon rounds up, and for a time two nucleoli are present (fig. 25). The absence of any evidence of disintegration in these nucleoli, combined with the fact that in later stages the fusion nucleus contains a single large nucleolus, indicates that the two bodies soon fuse.

The fusion nucleus assumes at once the resting condition (fig. 26), a delicate reticulum being formed. It undergoes also a pronounced increase in size, and in this and subsequent stages stains sharply. As it prepares to pass from the resting condition into mitosis, the chromatin granules fuse to form larger masses at the interstices of the network (fig. 27). These larger masses then gradually take on an elongated shape, and a definite thread is formed. This is thrown into definitely thickened loops which appear to be eight in number (fig. 28). Since the diploid chromosome number is eight, these loops probably represent the chromosomes. The spirem condition (fig. 29) which thus results is striking in appearance, and the large number of nuclei at this stage in the preparations show it to be of relatively long duration.

The spirem gradually passes to one side of the nucleus (figs. 30, 31), and finally contracts into a typical synaptic knot (fig. 32). In some cases the nucleolus is caught in this (figs. 31, 32), in others it lies free in the other half of the nucleus (fig. 30). The nucleolus shown in figure 32 stands out clearly as a red sphere within an enveloping tangle of blue chromatin. In figure 33 a nucleus is shown in which the spirem has apparently undergone longitudinal splitting. Nuclei presenting this appearance are uncommon in the preparations, and the writer is in doubt concerning the point. In subsequent stages the spirem segments (fig. 34), and the segments shorten into chromosomes. The nucleolus persists throughout all stages of mitosis, and passes into one of the daughter nuclei in late telophase.

The spindle (figs. 35, 36) resembles in shape and general appearance those which are formed in the conjugate divisions in the hyphae of the sporophore but is larger. The achromatic fibers form a well defined

bipolar spindle, and at each pole the centrosome appears as a deeply staining point (figs. 35, 36). Astral radiations have not been observed. The chromosomes are somewhat elongated, rod-like bodies, and when they occupy the equatorial region can be counted with certainty. They do not scatter as they migrate toward the poles as in the conjugate divisions, but are drawn apart in two well defined groups. The spindle at the beginning of the division is definitely intranuclear, but the nuclear membrane soon breaks down, and at late telophase the two groups of chromosomes lie free in the cytoplasm with indications of spindle fibers between them (fig. 37).

The daughter nuclei soon round up, assume a definite membrane, and a nucleolus appears in each (fig. 38). At the completion of the mitosis the basidium undergoes rapid elongation, and the two nuclei migrate apart. The basidium soon attains its full length, and the nuclei enter the second mitosis.

Few basidia have been found undergoing the second nuclear division, and it probably consumes far less time than the first. The spindle in this second mitosis is smaller than that in the first but resembles it in all other respects (fig. 39). The chromosome number is clearly four. The divisions in the two nuclei are not necessarily exactly simultaneous. In figure 39 the upper nucleus shows the two groups of chromosomes passing toward the poles, while in the lower nucleus the separation has not yet occurred.

Before the completion of the second division a transverse septum begins to form near the center of the basidium, and when the daughter nuclei have rounded up the basidium is composed of two binucleate cells (fig. 40). When one of the nuclei resulting from the first mitosis divides more rapidly than the other, a second septum may be laid down in one of these cells before it appears in the other (fig. 41). Usually, however, these two septa are formed simultaneously so that the typical four-celled basidium results (fig. 42). Maire (36) figures and describes in *Auricularia mesenterica* similar cases in which the two septa last formed are laid down independently because of the more rapid division of one of the two nuclei.

Comparatively few basidia in the preparations show stages following the spirem condition of the fusion nucleus, and preceding the four-celled basidium. It is evident that the mitoses are completed and the septa laid down in a relatively short space of time. Four-celled basidia which have not yet begun to form sterigmata are numerous in

many of the sections. The nuclei in the four-celled basidium are all of the same size, and are considerably smaller than the fusion nucleus. The size varies little in different basidia and is maintained in the spore.

Sterigmata and Spore Formation

In the young condition the basidia stand at right angles to the surface of the sporophore and form a more or less definite palisade layer. During their elongation they fall over and assume a procumbent position. An examination of mature basidia shows that the resulting bend usually takes place in the basal cell of the basidium (figs. 42–45) rather than in the hypha which bears it. This procumbent position results naturally from the lack of rigidity in the long, slender, flexuous basidium, but is nevertheless of decided importance in that it allows the sterigmata to arise at right angles to the surface of the sporophore unhindered by contact with neighboring basidia or sterigmata. The sterigmata are developed consequently in a palisade layer almost as well marked as that of the young basidia. In fact, when both young and old basidia lie close together, as is commonly the case, the palisade may be composed of a mixture of these two structures.

Each cell of the basidium gives rise to a single long, cylindrical, flexuous sterigma (figs. 44, 45), which bears at its tip an elongated, more or less crescent-shaped spore. The sterigma frequently reaches a length two-thirds that of the mature basidium, and has a diameter approximately the same as that of its nuclei. In rare cases sterigmata may arise simultaneously from all the cells, but far more frequently they originate independently of one another. In some cases the apical cell is the first to bud, in others it is the last. As the sterigma pushes outward, the cytoplasm in the cell behind becomes increasingly vacuolate, and finally the basidium is entirely emptied. The nuclei in the various cells pass outward with the cytoplasm into their respective sterigmata. In some cases the nucleus passes out relatively early, in other cases it remains in the basidium until a sterigma of considerable length has formed. Before its passage outward it is globose, but in the tube it becomes somewhat elongated. This elongation is probably due in large measure to the stress exerted upon the membrane by the flowing cytoplasm. It is not due to the narrowness of the sterigma, since in some cases, in which marked elongation occurs, the diameter of the sterigma exceeds even the long diameter of the nucleus.

As the sterigma pushes outward, its tip is broad and rounded (figs. 43–45), but on reaching its full length it becomes acuminate (fig. 46). The production of a minute globose body at the tip marks the beginning of spore formation (fig. 47). This structure, termed the spore "initial" by Levine (32), gradually increases in size and elongates, the cytoplasm of the sterigma flowing out into it through the very narrow canal which results (figs. 48–56). While the young spore is forming at the tip of the sterigma the nucleus lies remote from this point. There is no evidence to show that it influences directly the formation of the spore "initial." As the cytoplasm flows outward the nucleus is carried along with it, and on reaching the narrow canal at the tip of the sterigma becomes greatly elongated, the diameter of the normal nucleus being many times that of the canal. The nucleus is, in fact, drawn out into a long rod, and all trace of the nuclear membrane is lost (figs. 51–54). The nuclear material at this stage stains deeply, and the rod has an irregularly beaded appearance. The size of the spore at the time of the entrance of the nucleus varies, but it has in most cases reached at least half its mature length. The nucleus after its entrance into the spore remains in some cases for a long time in the rod-like condition. Finally it contracts into an irregularly globose, homogeneous, deep-staining mass (fig. 55), which soon takes on a nuclear membrane and assumes the characters of a normal globose, resting nucleus (fig. 56). The development of the narrow canal and the assumption by the nucleus of the irregular rod-like form recall similar phenomena described by Levine (32), Maire (36), Petri (44), Fries (13), and other workers for various higher Basidiomycetes. There is no reason to believe, however, that in *Eocronartium muscicola*, as in some of these cases, the centrosome is involved in forming the sterigma or in directing the course of the nucleus through the canal into the spore. The evident fibrillar strands which Levine (32) describes in Boletus as extending from the centrosome in the spore "initial" down through the canal to the definitely beaked nucleus in the basidium have no counterpart in Eocronartium. The passage of the nucleus into the sterigma is accomplished simply by the outward flow of the cytoplasm, and its passage through the canal into the spore is evidently of similar nature, no pull by kinoplasmic strands being exerted. In a unicellular basidium, as in Boletus, there is evidently need of a specialized apparatus for directing the course of the different nuclei into their respective sterigmata, since without such a

controlling apparatus one spore might receive two or more nuclei and another none. The formation of septa in *Eocronartium muscicola* eliminates this possibility.

Since a large number of nuclei in the rod-like condition are present in the preparations, it is evident that considerable time is consumed in the passage from the sterigma into the spore. This results from the great length of the nucleus, and probably also from the fact that the denser nature of the nucleus retards its flow through the canal.

After the passage of all the cytoplasm into the spore (fig. 57), the spore is freed from the sterigma. The writer has not studied the phenomena attending the liberation of the spores, and cannot say whether or not they are forcibly discharged as in many other Basidiomycetes. The mature spore contains a single globose nucleus. A binucleate spore has never been observed.

Spore Germination

In wet weather spores frequently begin to germinate on the sporophore where they have fallen among the basidia (fig. 59). They can be induced to germinate very readily in the laboratory in tap-water or in synthetic nutrient solutions (figs. 60, 61). Germination takes place by the formation of one or more germ-tubes, and the spore germinates in the uninucleate condition. The division of this nucleus has not been observed, and germ-tubes containing more than one nucleus have not been found. The writer in his previous paper (12) on *Eocronartium muscicola* discusses in detail the phenomena exhibited in spore germination and figures all the stages obtained. The reader is referred to the sections of this paper on spore germination and inoculation experiments for a complete discussion of the problems encountered in the attempts to obtain later stages in spore germination. The failure to obtain these has rendered impossible the explanation of the origin of the binucleate condition of the mycelium in this species. Since the spore germinates in the uninucleate condition, and all the cells of the endophytic mycelium and sporophore ever observed are binucleate, it is probable that the binucleate series of cells arises in the germ-tube soon after germination, but they have not been observed. No clamp connections have been found either on the endophytic hyphae or in the sporophore. It is not impossible that they may be produced for a brief period on the young mycelium, but their absence on older threads renders such a supposition extremely doubtful. Consequently the re-

cent explanation of the maintenance of the binucleate condition in the Basidiomycetes advanced by Kniep (28) cannot be applied to *Eocronartium muscicola*. The conjugate divisions in this species certainly take place without the assistance of clamp connections. Moreover, clamp connections are never found on the basidia.

General Considerations

The discovery in the Uredinales of sexual cell fusions accompanied by a well defined alternation of generations leaves no room for doubt that in this group of the Basidiomycetes sexuality exists. The now familiar observations of Blackman (2, 3) and Christman (7, 8, 9) have been confirmed and amplified by investigations by Olive (39, 40, 41, 42, 43), Kurssanow (31), Hoffmann (21), Arnaud (1), Fromme (15, 16), Kunkel (29, 30), and others on various species and on special phases of the cytology of the group. The mass of evidence accumulated demonstrates that in the Uredinales a generation of uninucleate cells alternates with a generation of binucleate ones, the binucleate series arising by the fusion of two uninucleate cells, and the nuclear fusion which occurs universally in the mature teleutospore being followed in the promycelium by what is with reasonable certainty a numerical reduction of the chromosomes. Proof of conjugate divisions in the hyphae and in spore formation is undoubted. The positive results obtained have stimulated research on species in other orders of the Basidiomycetes. Comparatively little is known, however, of the closely related group, the Auriculariales.

Istvanffi (24) describes the germination of the basidiospores in *Auricularia Sambucina*, and figures a single spore transversely septate into two uninucleate cells, each giving rise to a cluster of curved, uninucleate conidia. He gives no other figures, and makes no further contribution to the cytology of the group.

Sappin-Trouffy (48), working with *Auricularia auricula-judae*, traces the nuclear history from the young basidium to the mature spore. He states that the fruit-body is composed of interwoven hyphae possessing frequent transverse septa, the cells, in many cases at least, being binucleate. He makes no effort to determine the point of origin of this binucleate condition, and fails to state definitely whether multinucleate or uninucleate cells occur. The basidia arise as terminal cells on the peripheral hyphae of the sporophore, and in the young condition are binucleate. The two nuclei in the basidium later fuse,

and the resulting fusion nucleus then increases rapidly in size. Later it divides, and the daughter nuclei migrate toward the ends of the now much elongated basidium. A transverse septum is then laid down. Subsequently these nuclei also divide and other septa are formed, the basidium being finally composed of four superimposed, uninucleate cells. From each cell a sterigma is then put out, and at its tip a spore begins to form. The spore after reaching maturity germinates in the uninucleate condition. In germination a secondary spore is developed, and the nucleus migrates into this. The germination of this secondary spore was not watched, and no later stages showing germ-tubes containing more than one nucleus were obtained. The nuclear divisions in the basidium were not actually observed, and no details of nuclear structures are figured or described. Branching, septate paraphyses composed of binucleate cells lie between the basidia. Sappin-Trouffy points out the resemblance between the transversely septate basidium of Auricularia and the internal promycelium of Coleosporium, but he lays little emphasis upon the point, and considers the basidium homologous with the oospore.

Juel (25), from the examination of another species, *Auricularia mesenterica*, gives a detailed account of the nuclear divisions in the basidium, but adds nothing to the knowledge of the nuclear history in this genus. He states that the fusion nucleus lies at the center of the cylindrical basidium and is of an elongated shape due to the narrowness of the cell. It contains an evident nucleolus and a delicate chromatin network. Without leaving its central position it undergoes mitosis, the nuclear membrane disappearing and the spindle lying parallel to the long axis of the basidium. Delicate astral rays may be seen at each pole radiating into the cytoplasm from a deeply staining point which seems to be a centrosome. On the spindle there are six or eight deep-staining bodies which Juel regards as chromosomes. In the second division in the basidium the spindles are smaller and stouter, and lie within a well defined nuclear membrane. They are in all other respects similar to the spindle of the first division, and resemble it in lying parallel to the long axis of the cell. Juel advances the theory that the Basidiomycetes are phylogenetically of two groups, "the Protobasidiomycetes (Uredinales, Auriculariales, and Dacryomycetales) and the Autobasidiomycetes (Tremellales and Hymenomycetales)," in the former the spindle lying parallel to the long axis of the basidium, and in the latter at right angles to it.

Maire (36), having re-examined the species (*Auricularia mesenterica*) studied by Juel, states the chromosome number to be two. He terms the bodies figured by Juel protochromosomes, and maintains that two is the constant haploid chromosome number in all the Basidiomycetes (Ishikawa, 23; Tischler, 50). His figures show that after the division of the fusion nucleus in this species no septum is laid down until the spindles of the second division are already formed. A two-celled basidium exists for a very brief period. Since the daughter nuclei of the fusion nucleus do not always divide simultaneously, the two septa last formed are occasionally laid down independently. A case of this kind, in which the basidium contains only two of its three septa, is figured.

None of these investigators describe conjugate divisions in the hyphae. They do not even show that a binucleate condition is a constant characteristic of any definite portion of the life cycle. Their accounts of the nuclear divisions in the basidium are contradictory, and nuclear phenomena following spore germination are not described.

Our knowledge of the nuclear history and general cytology of the higher Hymenomycetes and Gastromycetes is also still far from satisfactory. The basidia in practically all described cases are binucleate in the young condition, and arise from binucleate cells in the subhymenium. Other cells in the hyphae of the sporophore or in the nutritive mycelium may be uninucleate or multinucleate. The accounts of different investigators differ greatly with respect to the point of origin of the binucleate condition in different species. The bulk of the evidence seems to show that the nuclear pairs do not arise at any given point or in any specialized manner. Recently Kniep (28), working with *Corticium varians* Kniep and *C. serum* Pers., has reached the conclusion that the binucleate condition is initiated and maintained by means of clamp connections. He points out also that the basidium is frequently connected by a clamp connection with the cell below, and he presents an interesting argument to show that the basidium is homologous with the ascus. He likens the formation of the clamp connection on the basidium to crozier formation on the ascogenous hypha, and homologizes the terminal cell of the clamp with that of the ascus hook. His work is extremely interesting since it furnishes the most plausible explanation yet advanced of the function of the clamp connections in the Basidiomycetes. His theory fails, however, to explain the large number of described cases

in which the basidia are formed without clamps (Levine, 33). Moreover, it fails to explain how the binucleate condition arises in species lacking clamp connections. He promises to elucidate these latter cases in a further contribution.

It is difficult to apply Kniep's explanation of the origin of the binucleate condition to *Eocronartium muscicola*. If clamp connections occur in this species for a brief period following spore germination, it does not seem logical to suppose that they would function as described by Kniep for a few cell generations and then cease to be developed on all the older mycelium. It seems probable to the writer therefore that clamp connections are wholly absent in *Eocronartium muscicola*.

Since the binucleate series in the Uredinales is initiated by a simple cell fusion, the discovery of a similar phenomenon in members of the Auriculariales would not be unexpected. Possibly such a fusion occurs in *Eocronartium muscicola* at a point in the life cycle following soon after spore germination. Since many facts in connection with this fungus indicate its close relationship with the rust fungi, this is a reasonable hypothesis. The investigation of the cytology of other members of the Auriculariales is very desirable in this connection. It is possible that other species present more favorable material for the determination of the origin of the binucleate condition than is available in *Eocronartium muscicola*. The determination of this point is of unusual interest because of its bearing on the phylogeny of the Uredinales.

Summary

1. The investigation of the cytology of *Eocronartium muscicola* is based on material from a single host, *Climacium americanum*, collected in the vicinity of Ithaca, N. Y.

2. The mycelium of the parasite is intracellular and permeates throughout the host plant from the underground stolons to the tips of the erect gametophoric branches. All the cells of the mycelium in which the nuclear number has been determined are binucleate, and conjugate divisions occur regularly. Uninucleate or multinucleate cells have not been found.

3. The fungous sporophore arises at the tip of a gametophoric branch of the moss plant, and is formed by the outward growth of the endophytic hyphae. These hyphae pass out into the spaces between

the overlapping moss leaves and grow upward, sheathing the apical region and developing a clavate Typhula-like fruit-body.

4. The cells of the hyphae composing the sporophore are all binucleate.

5. The chromosome number in the conjugate divisions has been determined with reasonable certainty to be four. The nucleolus lies outside the spindle, and enters one of the daughter nuclei in telophase.

6. The young basidia stand at right angles to the surface of the sporophore, and are unicellular and binucleate. Later the pair of nuclei approach each other and fuse.

7. The fusion nucleus passes from the resting into the spirem stage, and later the thread contracts into a definite synaptic knot.

8. The spindle of the first division is intranuclear. It holds no definite position with reference to the long axis of the basidium. A definite centrosome appears at each pole, but no astral radiations have been noted.

9. The chromosome number in the first division is certainly four. As in the conjugate divisions the nucleolus enters one of the daughter nuclei.

10. With the rounding up of the two daughter nuclei the basidium increases greatly in length, and the nuclei migrate into the opposite ends of the cell. They are considerably smaller than the fusion nucleus.

11. The second mitosis is more difficult to study on account of the smaller size of the nuclei, but intranuclear spindles with centrosomes are formed. The two divisions are not always exactly simultaneous in the two nuclei.

12. The four nuclei which round up after the second division migrate apart, and transverse septa are laid down dividing the basidium into four approximately equal superimposed, uninucleate cells. The central septum is laid down first.

13. Each cell of the basidium puts out a long cylindrical sterigma into which passes all the cytoplasm and the nucleus of the basidial cell. The basidium does not produce sterigmata in all its cells simultaneously or in any definite order. The sterigma at maturity is sharp-pointed.

14. A minute, globose spore "initial" forms at the tip of the sterigma, and this develops rapidly into an elongate spore which at maturity is more or less definitely crescent-shaped. All the cytoplasm and the nucleus of the sterigma pass into the spore, the nucleus being

drawn out into a long, irregular, deep-staining rod in order to effect its passage through the narrow canal between the sterigma and the spore. No evidence is given to indicate that the centrosomes function in directing the nucleus into the sterigma or in pulling it into the spore. The elongate rod after its entrance into the spore soon contracts into a globose, homogeneous, deep-staining mass which later takes on the usual appearance of a globose resting nucleus.

15. The spore is liberated from the basidium in the uninucleate condition. Germination in wet weather frequently occurs at once on the sporophore. The spores may be induced to germinate in nutrient solutions and on solid media. In germination the cytoplasm and nucleus pass out into a germ-tube, but the nucleus never undergoes division there. There is no increase in the amount of cytoplasm, and septa are not formed.

16. Nothing is known of that phase of the nuclear history which follows spore germination and precedes the appearance of the binucleated series of cells in the endophytic hyphae. Consequently the origin of the binucleate condition has not been determined. The difficulties which have been encountered in the investigation of this phase of the life cycle are discussed in the writer's previous paper on *Eocronartium muscicola* (12).

DEPARTMENT OF PLANT PATHOLOGY,
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EXPLANATION OF PLATES XXX–XXXII

All the figures were drawn with the aid of a camera lucida. A Zeiss 2 mm. apochromatic oil immersion objective (1.4 N.A.) and an 8 compensating ocular were used. As reproduced the figures represent a magnification of about 2150.

FIGS. 1–4. Endophytic hyphae of *Eocronartium muscicola* in the tissue of *Climacium americanum*. The host cells are merely outlined.

FIG. 1. A fungous hypha showing a single extremely long binucleate cell. The deep-staining bodies at the upper end are cytoplasmic granules. The deep-staining pads on the transverse septa in this and other figures probably indicate the presence of protoplasmic connections. The large, much-elongated host cells are characteristic of the main axis of the moss gametophore.

FIG. 2. A much shorter cell with more minute nuclei; in the same general region of the host.

FIG. 3. Terminal cell of a hypha passing from the interior of a gametophoric branch out into the space beneath one of the leaves. The leaf is shown in section at the right. The tip of the hypha has turned upward toward the apex of the host branch where later it would unite with other hyphae to form the sporophore.

FIG. 4. Terminal cell of a hypha in the apical region of the host.

FIGS. 5–19. Hyphae in the interior of the sporophore.

FIG. 5. Unusually short cells with minute nuclei. The cells are binucleate and the pads on the septa are prominent.

FIG. 6. A very long cell in an adjacent hypha. The cell contains two large nuclei, and numerous deep-staining cytoplasmic granules.

FIG. 7. A pair of nuclei in a cell of one of the hyphae of the sporophore. They are in the spirem condition, possibly near synapsis.

FIG. 8. Another pair of nuclei much smaller in size and possibly in very early prophase. Each contains four deep-staining bodies resembling chromosomes.

FIGS. 9–16. Other pairs of nuclei in various stages of conjugate division.

FIGS. 17–18. Four-nucleate cells; the two pairs of nuclei in each cell resulting from conjugate division.

FIG. 19. A terminal binucleate cell on a hypha at the periphery of the sporophore. Such a cell by division cuts off the young binucleate basidium.

FIGS. 20–45. Basidia.

FIGS. 20–23. Young binucleate basidia. There is considerable variation in shape.

FIG. 24. A young basidium in which nuclear fusion is taking place.

FIG. 25. A basidium in which the fusion nucleus still contains two nucleoli.

FIG. 26. Fusion nucleus at a stage near the resting condition.

FIG. 27. Fusion nucleus at a slightly later stage showing chromatin aggregated into larger masses at the interstices of the network.

FIG. 28. Spirem thrown into eight definitely thickened loops.

FIG. 29. Spirem loops less definite.

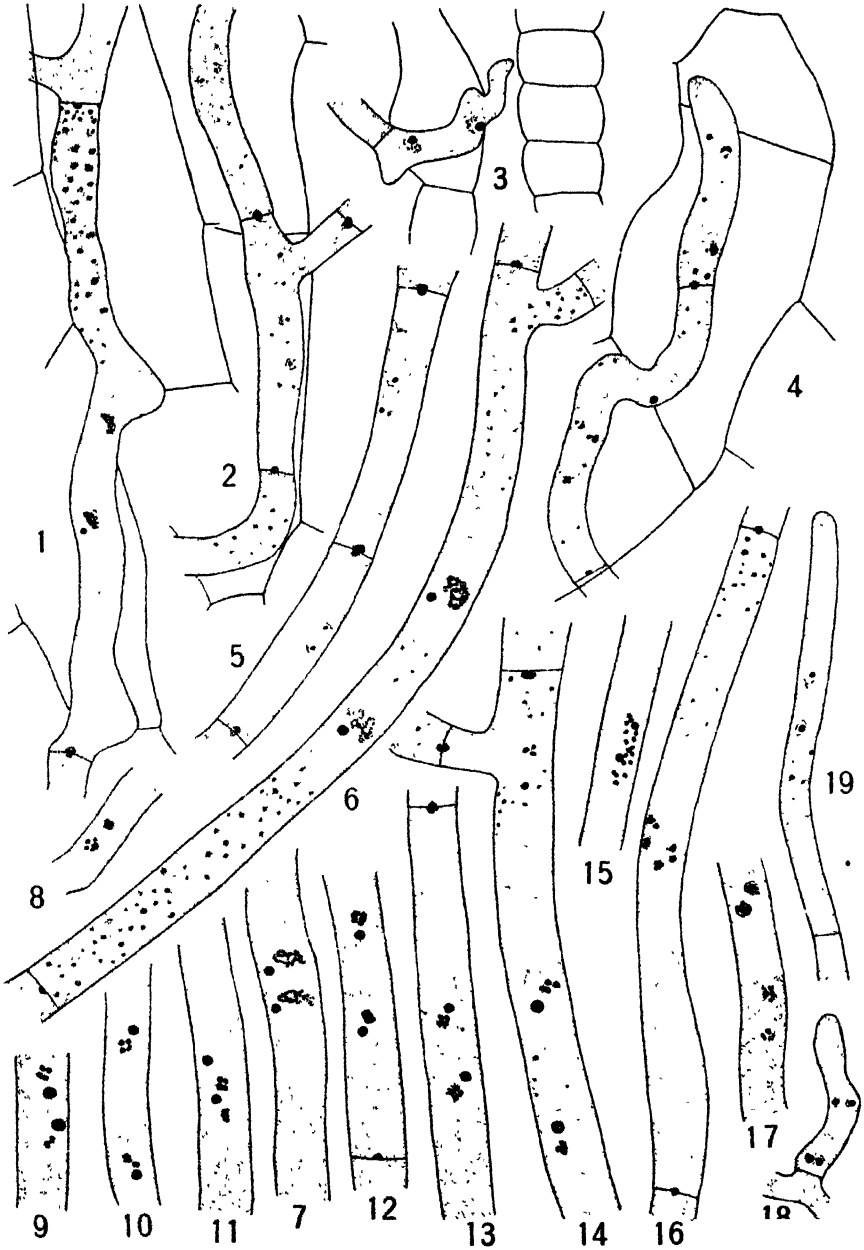
FIGS. 30, 31. Spirem at one side of nucleus, preceding synapsis.

FIG. 32. Synapsis; nucleolus enmeshed in the chromatin strand.

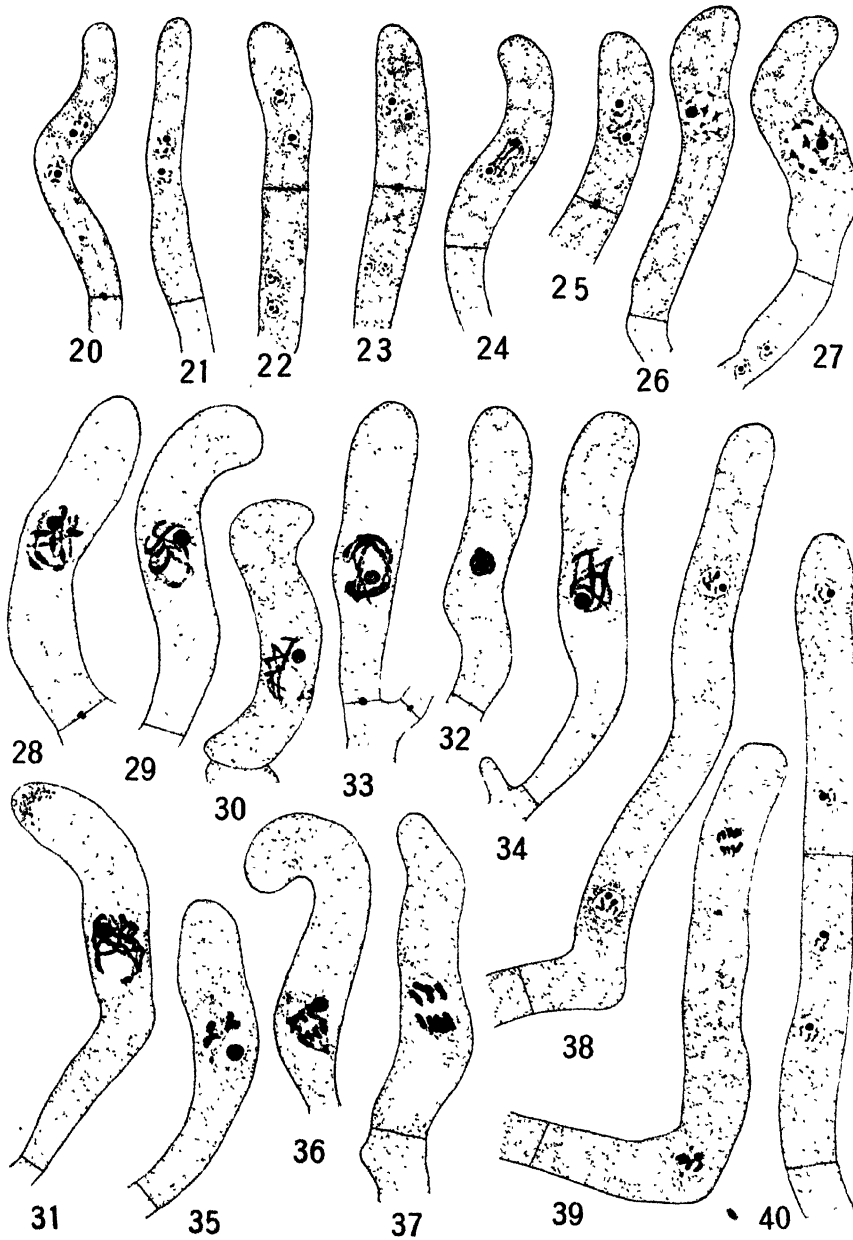
FIG. 33. A spirem giving some indication of a longitudinal split.

FIG. 34. A segmented spirem.

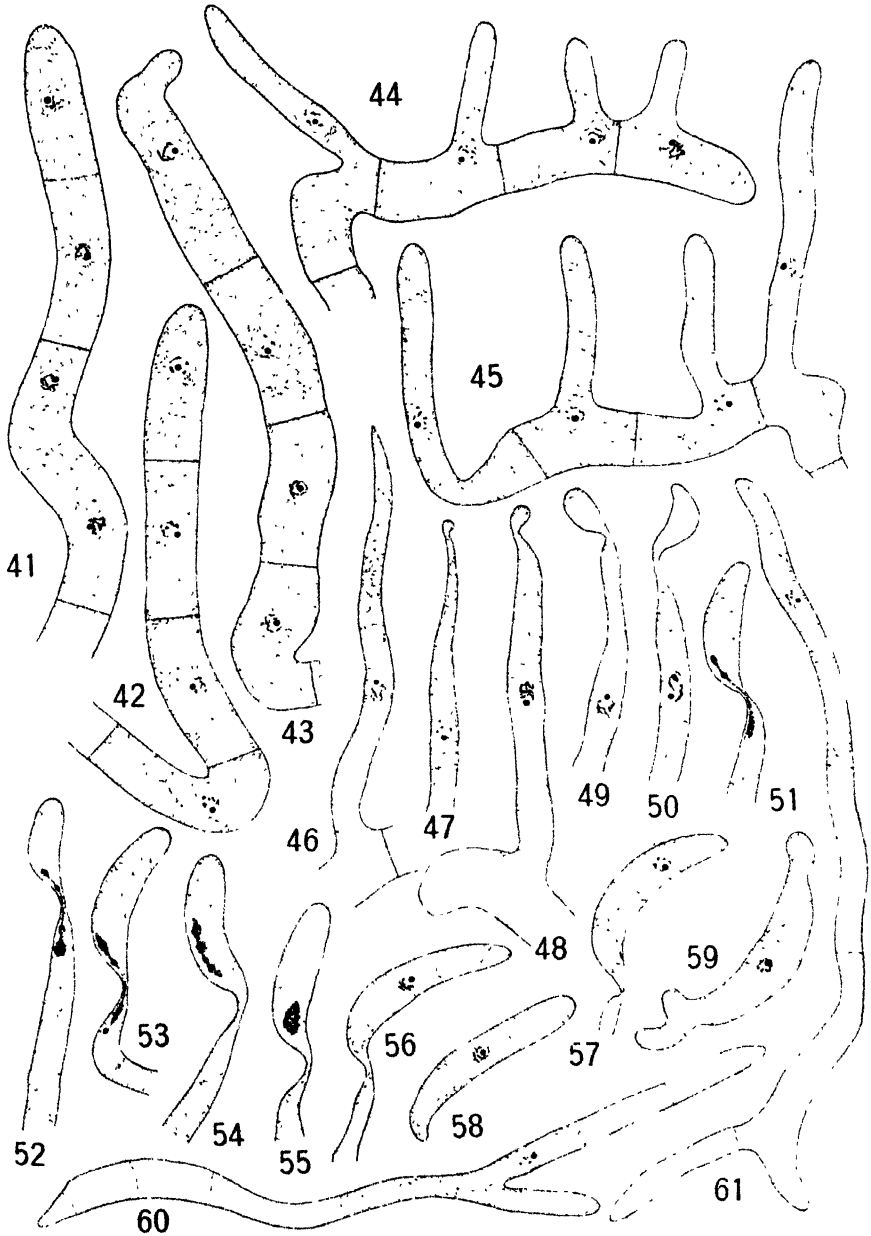
FIG. 35. First nuclear division showing four chromosomes; metaphase.



FITZPATRICK: CYTOLOGY OF EOCRONARTIUM.



FITZPATRICK: CYTOLOGY OF EOCRONARTIUM.



FITZPATRICK: CYTOLOGY OF EOCRONARTIUM.

FIG. 36. The chromosomes moving toward the poles; anaphase.

FIG. 37.* Telophase: nucleolus at one pole.

FIG. 38. An elongated, binucleate basidium.

FIG. 39. Second nuclear division, one nucleus dividing more rapidly than the other.

FIG. 40. A two-celled, four-nucleate basidium. The middle septum is always the first formed.

FIG. 41. A three-celled basidium. The second mitosis in this basidium was evidently not exactly simultaneous in the two nuclei. Consequently one septum is partially formed while the other has not yet appeared.

FIG. 42. A typical four-celled basidium.

FIG. 43. A mature four-celled basidium which is beginning to form a sterigma from the apical cell.

FIGS. 44, 45. Mature basidiá forming sterigmata.

FIGS. 46–58. Sterigmata and spore formation.

FIG. 46. A completely formed sterigma with an acuminate tip.

FIG. 47. Sterigma at the tip of which the spore "initial" is beginning to form.

FIGS. 48–50. Stages in the enlargement of the young spore. The sterigma in each case contains a normal nucleus.

FIGS. 51–53. The passage of the nucleus into the spore. It is drawn out into a long, irregularly beaded rod.

FIGS. 54–55. Stages in the transformation of the nucleus from the deep-staining rod into its normal form.

FIG. 56. Normal nucleus in a nearly mature spore.

FIG 57. Mature spore attached to the sterigma.

FIG. 58. Mature, detached spore.

FIGS. 59–61. Spore-germination.

FIG. 59. Early stage in spore germination; germ-tubes formed at both ends of the spore. This spore germinated on the sporophore in wet weather.

FIGS. 60–61. Spores induced to germinate in hanging drop culture. The apparent septa are merely dried hyaloplasm.

UREDINALES OF GUATEMALA BASED ON COLLECTIONS BY E. W. D. HOLWAY

II. Aecidiaceae, Exclusive of Puccinia and Form-Genera

J. C. Arthur

The first portion of this account of the Guatemalan rust flora was issued in a previous number of this journal (June, 1918, pp. 325–336), and listed the twenty-two known species belonging to the families Coleosporiaceae and Uredinaceae. For convenience in indexing, the species are numbered consecutively with the previous part. The portion here submitted and the parts to follow will deal with the family Aecidiaceae (Pucciniaceae). The genera Pucciniosira, Endophyllum, and the very similar Endophylloides, are usually placed with the Uredinaceae (Melampsoraceae), but it is believed that their affinities are better expressed in the present connection.

There are 79 species listed in this second portion, which fall into sixteen genera, all being small with one to three species each, except Ravenelia and Uromyces with twenty and thirty-five species respectively. The genus Ravenelia has its greatest development in the tropics, and the addition of five new species at this time indicates that many more new forms are yet awaiting discovery.

Probably the most interesting and striking new species of the eleven that are included in the paper is the one which introduces the genus Skierka to the flora of the western hemisphere. The whole morphological structure is unusual, and the long, flexuous filaments of agglutinated urediniospores, after the fashion of Uredinopsis, give to the specimen an astonishingly close resemblance, when seen with the naked eye or a hand lens, to the telia of Cronartium.

The species next in interest is *Dicheirinia binata*, for although the name was published sixty years ago the identity of the host has remained wholly conjectural until now, not even the family having been correctly suspected. It was originally collected in Nicaragua, a country adjacent to Guatemala. Recent collections were passing under the name *Uredo Cabreriana*.

Family: **Aecidiaceae (Pucciniaceae)**

23. RAVENELIA INGAE (P. Henn.) Arth. (on Mimosaceae).

Inga edulis Mart., Chinautla, Dept. Guatemala, Feb. 12, 1916, II_2, *486*; San Felipe, Dept. Retalhuleu, Jan. 14, 1917, 0, II_1, II_2, *719*.

In studying the 1917 collection (no. 719), very large urediniospores were encountered, 37–55 μ long, which were longitudinally striate or rugose and also reticulated. There also occurred smaller, echinulate urediniospores, 18–23 μ long, corresponding to those described in the North American Flora (7: 133). Recently the writer erected a new species of Inga rust (*R. Whetzelii* Arth., Mycol. 9: 64. 1917), in which the urediniospores are echinulate-verrucose and longitudinally striate, and are 30–40 μ long. As there seemed to be mixtures of several forms on the Guatemalan collections, a careful reexamination of all material at hand was undertaken, the work being carried out by Dr. E. B. Mains.

It was soon noticed that in previous descriptions and discussions, although pycnia were observed, there had been no discrimination between primary and secondary uredinia. Upon studying the primary and secondary forms separately, it was found that the latter had quite uniformly small, echinulate spores, while the former had much larger spores, very variable in size and sculpturing. The primary form, accompanied by pycnia, causes slight or no hypertrophy, while the secondary form, unaccompanied by pycnia, produces considerable hypertrophy, especially in the young caulicular parts. The type material for *Uredo Ingae* P. Henn. consists of secondary uredinia, while that for *U. excipulata* Syd., *R. Ingae* Arth., and *R. Whetzelii* Arth., is largely primary in each case, all now believed to represent variations of one species. Twenty collections have been studied, including all the types, from which Dr. Mains has drawn up the following emended description:

Pycnia amphigenous, numerous in crowded groups 1–3 mm. across, depressed hemispherical, subcuticular, dark brown, 85–160 μ broad by 25–65 μ high.

Primary uredinia amphigenous, causing no or slight hypertrophy, circinating about the pycnia in areas 1–6 mm. in diameter, somewhat tardily naked, pulverulent, dark cinnamon-brown, subepidermal, ruptured epidermis conspicuous; urediniospores variable in size and shape, obovoid, clavate, or obovoid-fusiform, 15–26 by 23–55 μ, usually large, 32–40 μ long or sometimes very large, 37–55 μ long; wall golden-brown, 1.5–4 μ thick, thicker at apex, 3–10 μ, prominently

striate or rugose longitudinally with more or less evident reticulations, especially noticeable on the large spores, sometimes verrucose-striate above and nearly or quite smooth below, the pores 3 or 4, equatorial.

Secondary uredinia amphigenous and caulicolous, often covering and deforming the leaf stalks and young shoots, confluent on the leaves in irregular patches, 0.5–2.5 cm. across, early naked, highly pulverulent, cinnamon-brown, ruptured epidermis conspicuous; urediniospores broadly ellipsoid or obovoid, 13–19 by 18–26 μ; wall golden-brown, 1.5–2 μ thick, thicker above, 3–5 μ, moderately or sparsely echinulate, the pores 3 or occasionally 4, equatorial.

Telia unknown.

24. RAVENELIA ENTADAE Lagerh. (on Mimosaceae).

Entada sp., Mazatenango, Dept. Suchitepequez, Feb. 22, 1916, II, *517*.

The species has heretofore been known only from the type collection, made by Lagerheim in Panama.

25. RAVENELIA SILIQUAE Long (on Mimosaceae).

Vachellia Farnesiana (L.) W. & A. (*Acacia Farnesiana* Willd.), Laguna, Lake Amatitlan, Feb. 8, 1915, II, *199*; Agua Caliente, Dept. Guatemala, Feb. 10, 1917, II, *850*.

Neither primary uredinia nor telia have yet been discovered for this rust, although it is not uncommon throughout southern Mexico, Central America, and the West Indies.

26. RAVENELIA LEUCAENAE-MICROPHYLLAE Diet. (on Mimosaceae).

Acacia angustissima (Mill.) Kuntze (*A. filicina* Willd., *A. filiculoides* Trel.), Guatemala City, Jan. 1, 1915, ii, III, *9*; Solola, 5100 feet alt., Jan. 27, 1915, ii, III, *138*; Panajachel, Dept. Solola, Jan. 3, 1917, II, III, *674*.

Two of these collections, nos. 138 and 674, were transmitted by Professor Holway with the host given as Leucaena, while another packet of no. 674 was transmitted later with the host given as Acacia, the determination being supplied by Mr. Paul C. Standley of the National Museum. No. 9 had the host named in 1915 by Mr. Standley as *Acacia filicina* Benth. Nos. 9 and 138 are accompanied by full-sized seed pods.

There appear to be no differences between this set of collections and the Holway collection of *Ravenelia Leucaenae-microphyllae* from Mexico. The foliage of all these collections is remarkably similar. The fruit has been seen for only two of them, nos. 9 and 138. It is

probable that the fragmentary collection which served as the type of the species is really an Acacia and not Leucaena. The type shows only a few imperfect urediniospores, while the present collections have well developed uredinia.

The uredinia are hypophyllous, on purple spots, round, 0.2–0.5 mm. across, early naked, somewhat pulverulent, cinnamon-brown, with the ruptured epidermis evident. The urediniospores are oblong or elongated ellipsoid, 13–16 by 26–35 μ, the wall cinnamon-brown, 1–1.5 μ thick and somewhat thicker above, about 3 μ, moderately echinulate, with 4 equatorial pores. The few spores heretofore seen did not show the thickening above, or the true form and size. The paraphyses intermixed with the spores are erect, clavate-capitate, 10–15 by 50–77 μ, with the wall chestnut-brown above and colorless below, and from 0.5 μ thick at the sides to 3–5 μ thick at the apex.

The species differs from *R. australis* Diet. & Neg., and from *Uredo Hieronymi* Speg., both on *Acacia Farnesiana*, in the paraphyses, and also in the urediniospores. Long[1] has recently found *R. australis* in Texas, and gives a detailed account of the species. The two species are similar in many respects, as noted in the North American Flora (7: 134), but the urediniospores of *R. Leucaenae-microphyllae* are more slender, and the paraphyses are erect and capitate, not at all incurved and hyphoid as in *R. australis*.

27. RAVENELIA IGUALICA Arth. (on Mimosaceae).
Acacia angustissima (Mill.) Kuntze (*A. filicina* Willd., *A. filiculoides* Trel.), Quezaltenango, Jan. 21, 1915, III, *97*; Solola, 7000 feet alt., Jan. 25, 1915, II, III, *119*.

The species has been taken heretofore but a few times between Texas and southern Mexico.

28. **Ravenelia inquirenda** Arthur & Holway sp. nov. (on Mimosaceae).
Acacia bursaria Schrenck, Laguna, Lake Amatitlan, Feb. 8, 1915, II, *196*.

Uredinia amphigenous, scattered or somewhat grouped, roundish, 0.1–0.3 mm. across, somewhat tardily naked, subepidermal, opening by a slit or pore, pulverulent, cinnamon-brown, ruptured epidermis conspicuous; paraphyses intermixed with the spores, cylindric or clavate-capitate, 7–10 by 26–42 μ, the wall cinnamon-brown, uniformly about 0.6 μ thick; urediniospores ellipsoid or obovoid, 15–18 by

[1] Bot. Gaz. 64: 65. 1917.

23–29 μ; wall cinnamon-brown, moderately thick, 1.5–2 μ, moderately echinulate, the pores 4, equatorial.

Telia unknown.

This rust appears to differ from all species so far described, and although without telia, requires to be independently named. Of the various Acacia-Leucaena-Calliandra rusts this is most like *R. gracilis* Arth., first reported from Mexico and recently found by Long in Texas (Bot. Gaz. **64**: 66), but has smaller spores, with fewer pores.

29. **Ravenelia distans** Arthur & Holway sp. nov. (on Mimosaceae).

Genus and species undetermined, Retalhuleu, Feb. 26, 1916, ii, III, *535*.

Urediniospores in the telia, lance-ovoid, 12–15 by 19–26 μ, usually acute above, somewhat narrowed below; wall cinnamon-brown, thin, 1 μ, much thicker above, 3–7 μ, moderately echinulate, the pores 4, equatorial.

Telia hypophyllous, scattered, round or oblong, 0.2–0.5 mm. across, early naked, subepidermal, chestnut-brown, ruptured epidermis evident; teliospore-heads chestnut-brown, 4–6 cells across, 55–75 μ in diameter, each spore with 6–8 semihyaline spines, about 3 μ long; cysts adnate to the lower side of the marginal cells.

This species has urediniospores of characteristic form. It is close to *R. Patschkeana* Diet. on Calliandra from Brazil, but has smaller urediniospores and larger teliospore-heads composed of more spores. The leaves of the host have much the appearance of those of Mimosa.

30. **Ravenelia bizonata** Arthur & Holway sp. nov. (on Mimosaceae).

Calliandra Houstoni Benth., Guatemala City, March 16, 1916, II, iii, *584* (type).

Calliandra sp., Huehuetenango, Jan. 22, 1917, II, III, *762*.

Uredinia epiphyllous, scattered or somewhat grouped, round or oval, 0.2–0.4 mm. across, early naked, subcuticular, pulverulent, dark chestnut-brown, ruptured cuticle inconspicuous; paraphyses intermixed with the spores, capitate or clavate-capitate, 15–19 by 23–48 μ, the wall golden brown above, colorless below, 1–1.5 μ thick, much thicker above, 7–10 μ; urediniospores ellipsoid or obovoid, 15–19 by 20–26 μ; wall dark cinnamon-brown above, paler below, thin, 1–1.5 μ, sometimes slightly thicker above, very finely and closely echinulate below, usually smooth at apex, the pores 6–8 in two zones, one equatorial, the other subequatorial.

Telia usually epiphyllous, scattered, round, 0.1–0.3 mm. across, soon naked, subcuticular, chestnut-brown, ruptured cuticle noticeable; teliospore-heads hemispherical, 4–6 cells across, 55–75 μ in diameter, each spore with 2–4 colorless tubercles, 3–10 μ long; cysts attached to the lower side of each marginal spore.

The species appears to be closest to *R. mexicana* Tranz., a Mexican rust on Calliandra, which is yet, unfortunately, imperfectly known. It is also like *R. versatilis* (Peck) Diet., on *Acacia Greggii*, in its structure, but probably has little relationship to it.

31. RAVENELIA ECTYPA Arth. & Holw. (on Mimosaceae).
Calliandra gracilis Klotsch, Palin, Dept. Amatitlan, Dec. 24, 1916, II, III, *633*.
Calliandra sp., Laguna, Lake Amatitlan, Feb. 8, 1915, ii, III, *204*.
The species also occurs in Costa Rica on *C. gracilis*.

32. **Ravenelia sololensis** Arthur & Holway sp. nov. (on Mimosaceae).
Lysiloma acapulcensis Benth.(?), Solola, 7000 feet alt., Jan. 28, 1915, II, III, *147*.

Uredinia amphigenous and fruticolous, scattered, round or elliptic, 0.2–0.5 mm. across, on the fruit up to 4 mm. long, early naked, subcuticular, pulverulent, dark chestnut-brown, ruptured cuticle conspicuous; paraphyses intermixed with the spores, clavate-capitate, 13–16 by 64–87 μ, the wall colorless below, chestnut-brown above, 0.5 μ thick below, 3–4 μ thick above; urediniospores ellipsoid or broadly obovoid, 16–19 by 27–35 μ; wall light chestnut-brown above, paler below, 1.5 μ thick, sometimes a little thicker above, up to 3 μ, moderately echinulate, the pores 4, equatorial.

Telia amphigenous and fruticolous, scattered, round or elongated elliptic, 0.2–0.4 mm. across, on the fruit up to 6 mm. in length, early naked, subcuticular, dark chestnut-brown, ruptured cuticle conspicuous; teliospore-heads chestnut-brown, 7–9 cells across, 70–107 μ in diameter, each spore with 4–6 nearly colorless spines, about 3 μ long; cysts attached beneath the head.

The species differs noticeably from *R. Lysilomae* Arth. by having the teliospore-heads with spines instead of smooth, and in the differently shaped urediniospores. It has some resemblance to *R. Leucaenae* Long, but is abundantly distinct.

33. RAVENELIA ACACIAE-PENNATULAE Diet. (on Mimosaceae).
Acacia pennatula Benth., Panajachel, 5100 feet alt., Dept. Solola, Jan. 30, 1915, II, iii, *162*.
Heretofore this species has been known only from collections made by Professor Holway in southern Mexico.

RAVENELIA MIMOSAE-ALBIDAE Diet. (on Mimosaceae).
34. *Mimosa albida* H.B.K., Solola, 5100 feet alt., Jan. 27, 1915, II, *137*.
The species, chiefly known from Mexico, was collected by Keller-

man on *M. albida floribunda* Robins., between Antigua and Volcan de Agua, Feb. 18, 1905, II, *5360*, and reported by Kern in Mycologia, *l. c.*

35. **Ravenelia Mainsiana** Arthur & Holway sp. nov. (on Mimosaceae).
Mimosa albida H.B.K., Guatemala City, Jan. 3, 1915, ii, III, *13*.

Uredinia amphigenous, scattered, oval, 0.2–0.8 mm. long, early naked, pulverulent, cinnamon-brown, ruptured epidermis evident; paraphyses intermixed with the spores, clavate or capitate, 7–16 by 29–45 μ, the wall slightly tinted, uniformly thin, 0.5–1 μ, the stipe often solid; urediniospores ellipsoid or broadly obovoid, 16–18 by 18–23 μ; wall cinnamon-brown, 1.5–2 μ, moderately echinulate, the pores rather indistinct, 8–10, scattered.

Telia amphigenous, scattered or in small groups, round or oval, 0.4–0.8 mm. across, subepidermal, soon naked, blackish, ruptured epidermis conspicuous; teliospore-heads irregular, flat, dark chestnut-brown, 3–6 cells across, 55–71 by 74–93 μ, each spore bearing 7–9 spines, up to 3 μ long; cysts pendent from base of pedicel, swelling and bursting in water.

This rust differs from *R. Mimosae-albidae* Diet. in well marked characters. The teliospore-heads are flat and irregular, not hemispherical and regular, the urediniospores are somewhat smaller and are echinulate not verrucose, while the paraphyses are smaller, lighter-colored, and thinner-walled than in the other species.

The host was determined by Mr. Paul C. Standley of the National Herbarium, who also determined the host for *R. Mimosae-albidae* (no. 137).

The species is named in honor of Dr. E. B. Mains, assistant botanist in the Indiana Experiment Station of Purdue University, who detected the specific distinctions and has drawn up the diagnosis. Dr. Mains has also done a large share of the microscopic work on the other species of Ravenelia listed in this paper, and also has given much aid in the critical study of some of the species of other genera, in all of which he has displayed excellent judgment and a fine sense of diagnostic values.

36. RAVENELIA SPINULOSA Diet. & Holw. (on Caesalpiniaceae).
Cassia biflora L., Solola, Jan. 27, 1915, II, iii, *134*; San Lucas Toliman, Dept. Solola, Feb. 3, 1915, II, III, *182*; Guatemala City, Feb. 14, 1917, II, iii, *867*.
Cassia sp., San Rafael, Dept. Guatemala, Jan. 9, 1915, II, III, *44*; El Rancho, Dept. Jalapa, Feb. 13, 1915, II, III, *209*.
The paraphyses of this species are quite variable; often there are

more cylindrical ones in a sorus than capitate ones. The species was collected by Kellerman on *C. biflora*, at Gualan, Dept. Zacapa, Dec. 30, 1905, II, III, *5441*, and reported by Kern in Journ. Myc. *l. c.*, and issued in Sydow, Uredineen *2089*, and in Kellerm. Fungi Sel. Guat. 9.

37. RAVENELIA INCONSPICUA Arth. (on Caesalpiniaceae).
Caesalpinia exostemma Moc. & Sesse, Sanarate, Dept. Guatemala, Feb. 10, 1916, II, III, *476*.

Heretofore only the type collection of this species has been known, which was obtained by Professor Holway in Mexico on a host identified only as "Caesalpinia or Cassia." The present collection agrees with the type in all respects except that the paraphyses have the walls thinner at the sides and thicker above.

From the present study of the two collections, it becomes evident that the following modifications should be made in the original description of the uredinia: urediniospores 13-16 μ in diameter, the wall 1.5-2 μ; paraphyses thickened above, 3-9 μ.

38. RAVENELIA HUMPHREYANA P. Henn. (on Caesalpiniaceae).
Poinciana pulcherrima L.

A collection made by Kellerman at Gualan, Dept. Zacapa, Dec. 27, 1905, II, III, *5427*, and reported by Kern in Journ. Myc. *l. c.*, and also issued in Kellerman's Fungi Selecti Guatemalensis 8, and in Sydow's Uredineen *2088*. Apparently only one collection was made, although Kern reports the year as "1906," and on the label of Kellerman's exsiccati the number is given as "5727." It is a common rust of tropical America, wherever the host occurs.

39. RAVENELIA SIMILIS (Long) Arth. (on Fabaceae).
Brongniartia sp., San Felipe, Dept. Retalhuleu, Jan. 13, 1917, III, *706*.

The host formed a small tree in hedgerows, as seen by Professor Holway, but was not in flower or fruit. The species has heretofore been known only from central Mexico.

40. RAVENELIA INDIGOFERAE Tranz. (on Fabaceae).
Indigofera mucronata Spreng., Solola, Jan. 27, 1915, II, iii, *122*.
Indigofera suffruticosa Mill., Palin, Dept. Amatitlan, Dec. 24, 1916, II, *637*.

Indigofera sp., Antigua, Dept. Sacatepequez, Dec. 27, 1916, II, III, *640;* Panajachel, Dept. Solola, Jan. 3, 1917, II, III, *667*.

A very common rust both of insular and continental America.

41. RAVENELIA LONCHOCARPI Lagerh. & Diet. (on Fabaceae).

Lonchocarpus sp., Mazatenango, Dept. Suchitepequez, Feb. 22, 1916, II, *511*, *522*.

Although no material has been available with which to compare, yet the very unusually shaped urediniospores leave little doubt that the species in hand is the one described by Lagerheim and Dietel from Brazil in 1894. The rust also occurs in Cuba on *L. latifolius* H.B.K., but is not known to have been found elsewhere since the original collection was made. The Guatemalan collections show bullate hypertrophies, 3–8 mm. across, but no pycnia could be detected on them.

42. RAVENELIA APPENDICULATA Lagerh. & Diet. (on Euphorbiaceae).

Phyllanthus acuminatus Vahl, San Felipe, Dept. Retalhuleu, Jan. 12, 1917, II, iii, *699*.

Phyllanthus sp., Solola, Jan. 27, 1915, ii, III, *127*; Guatemala City, Dec. 21, 1916, III, *616*; Panajachel, Dept. Solola, Jan. 3, 1917, III, *671*.

The collection no. 699 shows a more pulverulent appearance in the telia than usual, although the type collection has also something of this tendency toward fragile pedicels. The species also occurs in Mexico.

43. DICHEIRINIA BINATA (Berk.) Arth. (on Fabaceae).

Erythrina glauca Willd.

The type collection for *Uredo Cabreriana* Kern & Kellerm., now known to be a synonym of this rust, was made by Kellerman, at Livingston, Dept. Izabel, Jan. 18, 1905, *5465*. The host was determined as *Buettneria lateralis*, and so reported by Kern in Journ. Myc. *l. c.*, but when collections of the same rust from Porto Rico came to hand in 1913, the collection was resubmitted to Mr. John Donnell Smith, who had named it in the first place, and under date of May 1, 1913, he replied . . . " I now perceive that it should have been referred to *Erythrina glauca* Willd."

The teliospores were recently found by Mr. H. R. Rosen on a specimen in the Arthur herbarium collected in 1906 on *Erythrina glauca* at Paso Real (Prov. Pinar del Rio), Cuba, by Arbaca and

O'Donovan. They agree in every way with those of the scanty type material from Nicaragua, and show that they are not "one slightly higher on the pedicel than the other," as given in N. Am. Flora 7: 147, but that the two spores of each pair are borne side by side. It now seems probable that the host of the Nicaraguan collection is *E. glauca*, or at least a species of the genus with similarly thick, glaucous leaves. The rust also occurs on *E. umbrosa* H.B.K. in Trinidad, W. I., where it was collected by J. B. Rorer, Oct. 21, 1916.

44. TRANZSCHELIA PUNCTATA (Pers.) Arth. (on Amygdalaceae).
Prunus sp., San Rafael, Dept. Guatemala, Jan. 7, 1915, II, *28*.

This long-cycle, heteroecious species was collected by Kellerman on *Amygdalus persica* L., at Antigua, Feb. 15, 1905, II, *5358*, and reported by Kern in Journ. Myc. *l. c.*, under the early name, *Puccinia Pruni-spinosae* Pers.

45. PHRAGMOPYXIS DEGLUBENS (Berk. & Curt.) Dietel (on Fabaceae).
Benthamantha cinerea (L.) Kuntze, Guatemala City, Dec. 20, 1916, II, iii, *607*.

Only a meager amount of the fungus was found. It differs slightly from collections made in Mexico upon other species of the same host-genus by the teliospores possessing a short apiculus and a less gelatinous layer in the wall.

46. UROPYXIS SANGUINEA (Peck) Magn. (on Berberidaceae).
Mahonia pinnata (Lag.) Fedde.

A collection of this widespread, long-cycle rust, having pycnia, uredinia, and telia, was made by Kellerman at Volcan de Agua, Dept. Sacatépequez, Feb. 15, 1905, II, *4624*, and reported by Kern in Journ. Myc. *l. c.*

47. **Uropyxis Crotalariae** Arth. sp. nov. (on Fabaceae).
Crotalaria sp.

Uredinia amphigenous, oblong or irregular, large, 0.5–1 mm. long, soon naked, pulverulent, cinnamon-brown, ruptured epidermis somewhat overarching and conspicuous; urediniospores ellipsoid or globoid, 18–26 by 23–30 μ; wall golden-brown to light yellow, 2–2.5 μ thick, moderately echinulate, the pores 6–8, scattered.

Telia chiefly epiphyllous, like the uredinia but smaller, 0.1–0.2 mm. across; teliospores globoid, 26–30 μ in diameter, the septum wanting; wall hygroscopic, the inner, firm portion dark chestnut-brown, 2–2.5 μ thick, the outer gelatinous layer yellow, swelling to 5–6.5 μ thick, the colorless cuticle sparsely verrucose; pedicel short, colorless, largely evanescent.

The collection selected for the type of the species was collected on an undetermined species of Crotalaria, by W. A. Kellerman, at Laguna, altitude 4000 feet, on Lake Amatitlan, Dept. Amatitlan, Jan. 17, 1906, II, iii, *5397*. Another collection by Kellerman, showing only uredinia, was collected on *C. maypurensis* H.B.K., a cultivated plant called by the natives "Chipilin," at Guanda Viejo near Guatemala City, Feb. 3, 1905.

This is the first species of Uropyxis with one-celled teliospores yet recorded.

48. UROPYXIS DALEAE (Diet. & Holw.) Magn. (on Fabaceae).

Parosela diffusa (Moric.) Rose, Palin, Dept. Amatitlan, Dec. 24, 1916, ii, III, *638*.

Parosela domingensis (DC.) Millsp. (*Dalea domingensis* DC.), Guatemala City, Jan. 8, 1917, II, *681*.

Parosela nutans (Cav.) Rose, Guatemala City, Dec. 21, 1916, ii, III, *612*.

An abundant species in Mexico, where it has been collected by Professor Holway and others, but is now first reported elsewhere.

49. CALLIOSPORA DIPHYSAE Arth. (on Fabaceae).

Diphysa robinioides Benth., Guatemala City, 5000 feet alt., Jan. 1, 1915, 0, III, *8;* Solola, Jan. 27, 1915, 0, III, *121a*; Panajachel, 5100 feet alt., Dept. Solola, Jan. 30, 1915, 0, III, *157*; Mazatenango, Dept. Suchitepequez, Feb. 22, 1916, 0, III, *521*; San Felipe, Dept. Retalhuleu, Jan. 14, 1917, 0, III, *716*.

Diphysa sp. (probably *D. robinioides* Benth.), between San Lucas Toliman and Patalul, Feb. 4, 1915, 0, III, *191*; Patulul, 6000 feet alt., Dept. Escuintla, Feb. 4, 1915, III, *195*.

A short-cycle rust. In no. 191 most of the teliospores are much lighter-colored and thinner-walled than heretofore seen, giving at first sight the appearance of a distinct species. They are apparently not immature spores, but the early stage in the development of the sorus, in which these less resistant spores arise for a time to be replaced later by the usual dark-walled form, able to withstand greater variation in conditions. The lighter-colored form may be described as having inner walls cinnamon-brown, 1.5–3 μ thick, with the outer gelatinous layer swelling only 1–2 μ thick in water. These collections also show that many teliospores are smaller than given in the original description, which should have the lower limit of breadth placed at 23 μ, and of length 35 μ.

The species was also collected by Kellerman on *Diphysa* sp., at Palmar, Dept. Quezaltenango, Feb. 11, 1906, 0, III, *5459*, and reported by Kern in Mycologia *l. c.*

50. CALLIOSPORA HOLWAYI Arth. (on Fabaceae).

Eysenhardtia adenostylis Baill., Panajachel, Dept. Solola, Jan. 30, 1915, 0, III, *161*; same, Jan. 3, 1917, 0, III, *666*.

In both the collections here listed a small proportion of light-colored spores, corresponding to those described under *C. Diphysae*, are to be found. They have the inner wall cinnamon-brown, 1–1.5 μ thick, with the outer gelatinous layer scarcely swelling in water.

51. PROSPODIUM LIPPIAE (Speg.) Arth. (on Verbenaceae).

Lippia asperifolia Rich., Moran, Dept. Amatitlan, Dec. 22, 1917, ii, III, *617*.

Lippia strigosa Turcz., Solola, 7500 feet alt., Jan. 28, 1915, II, III, *152*; Quezaltenango, Jan. 16, 1917, ii, III, *730*; Zunil, Dept. Quezaltenango, Jan. 28, 1917, II, III, *787*.

Lippia umbellata Cav., Volcan de Agua, Dept. Sacatépequez, March 4, 1916, II, III, *554*.

Lippia sp., Tecpan, Dept. Chimaltenango, Jan. 1, 1917, ii, III, *661*.

A long-cycle rust, for which the pycnia and primary uredinia have not yet been recognized. It is often listed as *Puccinia Lippiae* Speg. It was collected by Kellerman on *Lippia myriocephala* Cham. & Schl., at Laguna, Lake Amatitlan, Jan. 20, 1906, ii, III, *5451* in part, and reported by Kern in Mycologia *l. c.*

52. PROSPODIUM TUBERCULATUM (Speg.) Arth. (on Verbenaceae).

Lantana sp., Huehuetenango, Jan. 22, 1917, ii, III, *767*.

A long-cycle rust of both North and South America, for which the pycnia and primary uredinia are yet undescribed. It is frequently listed as *Puccinia tuberculata* Speg.

53. PROSPODIUM APPENDICULATUM (Wint.) Arth. (on Bignoniaceae).

Tecoma mollis H.B.K. (*Stenolobium molle* Seem.), Antigua, 5500 feet alt., Dept. Sacatépequez, Jan. 13, 1915, ii, III, *75*.

Tecoma Stans (L.) Juss. (*Stenolobium Stans* D. Don), Sanarate, Dept. Guatemala, Feb. 10, 1916, 0, II, *469*; Palin, Dept. Amatitlan, Dec. 24, 1916, 0, II, *639a*.

Tecoma sp., on the pods, Sanejarate, between Barrios and Guatemala City, Feb. 12, 1915, II, *207*; Panajachel, Dept. Solola, Jan. 3, 1917, ii, III, *664*.

A long-cycle species, having pycnia, primary and secondary uredinia, and telia. It is often listed as *Puccinia appendiculata* Wint. Nos. *469* and *639a* are the first collections in which pycnia and primary uredinia have been seen. The pycnia are amphigenous, and are crowded in small groups on discolored spots 1.5–3.5 mm. across. They are subcuticular, light chestnut-brown, broadly conical, 67–135 μ broad by 39–50 μ high. The primary uredinia are amphigenous, encircling the pycnia, round or somewhat oblong, 0.2–0.8 mm. across. The urediniospores are slightly larger, and with a more hygroscopic layer, than in the secondary form. Other characters for the two forms are the same in both.

54. NEPHLYCTIS TRANSFORMANS (Ellis & Everh.) Arth. (on Bignoniaceae).

Tecoma Stans (L.) Juss. (*Stenolobium Stans* D. Don), Sanarate, Dept. Guatemala, Feb. 10, 1916, 0, III, *467*; Palin, Dept. Amatitlan, Dec. 24, 1916, 0, III, *639*.

A short-cycle species, often listed as *Puccinia transformans* Ellis & Ev. .No. *639* also showed a small amount of *Prospodium appendiculatum*, 0, II, on some of the leaves, and is recorded under that species as no. *639a*.

55. PHRAGMIDIUM OCCIDENTALE Arth. (on Rosaceae).

Oreobatis trilobus (Seringe) Rydb., Quezaltenango, Jan. 31, 1917, ii, III, *813*.

This collection agrees closely with the species as heretofore known on *Rubacer parviflorum* in the western United States and Canada, but the lower part of the telial pedicels are more highly hygroscopic, in water usually swelling until they burst.

56. PHRAGMIDIUM SUBCORTICINUM (Shrank) Wint. (on Rosaceae).

Rosa cult., Antigua, Dept. Sacatépequez, March 4, 1916, ii, III, *544*; Malacatancito, Dept. Huehuetenango, Jan. 25, 1917, II, III, *778*.

The species appears to be rare in Guatemala, although it is cosmopolitan on cultivated roses, especially on those having the general characteristics of *Rosa gallica*.

57. PHRAGMIDIUM POTENTILLAE (Pers.) P. Karst. (on Rosaceae).

Potentilla sp., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, III, *572*; same, Dec. 29, 1916, II, III, *657*.

A cosmopolitan rust of temperate regions. This is the first record for Central America, where it is doubtless rare.

58. **Skierka Holwayi** Arth. sp. nov. (on Sapindaceae).

Thouinidium decandrum Radlk. (?), Sanarate, Dept. Guatemala, Feb. 10, 1916, ii, III, *475*.

Thouinidium sp., Agua Caliente, Dept. Guatemala, Feb. 10, 1917, o, II, III, *849* (type).

Pycnia amphigenous, solitary or few in small groups, noticeable, reddish-brown, subepidermal, discoidal, 416–448 μ in diameter, 96–128 μ high; ostiolar filaments apparently wanting.

Uredinia chiefly epiphyllous, encircling the pycnia in groups 1–5 mm. across, round, 0.1–0.2 mm. in diameter, flask-shaped in cross section, covered by the greatly thickened epidermis, through which the dehiscence is by a small pore, the spores at first cohering in loose columns, soon falling apart and giving the spots a pulverulent, cinnamon-brown appearance; urediniospores oblong-fusiform or ellipsoid-fusiform, 19–26 by 43–60 μ when in alcohol or dry; wall golden-brown, 2.5–3 μ thick when dry or in alcohol, in water the outer, hygroscopic layer paler, swelling up to 7–10 μ, the apex beaked, 7–9 μ long in alcohol or dry, 10–15 μ long in water, very finely and inconspicuously verrucose when dry, appearing smooth when wet, the pores obscure, probably 2, equatorial.

Telia hypophyllous, opposite the uredinia and similar to them; teliospores oblong-fusiform, 12–19 by 35–50 μ exclusive of the acute or filiform beak, the narrowed base with a distinct hilum, cohering in long columns 4–5 mm. long, 80–150 μ in diameter; wall colorless, or slightly yellowish, the inner layer 1 μ thick, the outer layer not noticeable in alcohol or dry, swelling in water to 3–9 μ and disintegrating, with the apex filiform, up to 100 μ, and likewise disintegrating, the base deciduous from the slender, inconspicuous pedicel, leaving a noticeable hilum.

A very unusual rust, having the appearance of a Cronartium. The teliospores, however, are borne singly on pedicels from a flat hymenium, and breaking away are extruded in a long filament of agglutinated spores, held together by the mucilaginous outer layer of the spore wall. The genus was established by Raciborski for two species found in Java, the type species being on Burseraceae and the other on Euphorbiaceae. Another species was added by Hennings on Sapindaceae from the Congo region of Africa. In these three species the spores of both stages are smaller than in the American form, and the urediniospores have more of the customary appearance of those of rusts in general. In the present species the urediniospores are large, and have an outer hygro-

scopic layer. They are extruded from the mouth of the sorus and adhere in filaments to some extent, much as the teliospores do.

Were it not for the agglutinating action of the outer coat of the spores and the dropping away from the pedicel of the teliospores, after the fashion of urediniospores, this rust would doubtless be called a species of Uromyces. The decision reached by the Sydows (Monog. Ured. 3: 331) to place the genus Skierka under the Aecidiaceae (Pucciniaceae) appears to be well founded. The fortunate discovery of pycnia with the American material, thus completing the life cycle, adds to the understanding of its relationship.

The author takes special pleasure in commemorating the extensive and fruitful explorations by Professor Holway, and his untiring devotion to botanical science, by dedicating this unique species of rust to him.

59. SPHENOSPORA PALLIDA (Wint.) Diet. (on Smilaceae).

Smilax sp., San Felipe, Dept Retalhuleu, Jan. 14, 1917, II, III, *718*; Progreso, on the Puerto-Barrios-Guatemala City Ry., Feb. 12, 1917, II, III, *859*.

This waxy-looking rust also occurs in Costa Rica and South America.

Both uredinia and telia are subepidermal. The manner of septation of the teliospore, by which the two cells are equally poised on the pedicel, and not to any extent superposed, appears to warrant the validity of the genus. The gross appearance of the waxy telia is very distinctive. Pycnia have not been seen, and the full life cycle is yet unknown. The germination of the teliospores appears to be apical.

60. BAEODROMUS EUPATORII Arth. (on Carduaceae).

Eupatorium Aschenbornianum Schauer, Chinautla, Dept. Guatemala, Feb. 12, 1916, *478*, *484*.

A short-cycle rust, heretofore known only from two collections made by Professor Holway in central Mexico.

61. PUCCINIOSIRA PALLIDULA (Speg.) Lagerh. (on Tiliaceae).

Triumfetta semitriloba L., Mazatenango, Dept. Suchitepequez, Feb. 21, 1916, *509*.

A short-cycle rust, very common in tropical America. It was collected by Kellerman on *Triumfetta* sp., at Guatemala City, Feb. 3, 1905, *4608*, and reported by Kern in Journ. Myc. *l. c.*

62. **Pucciniosira Eupatorii** Lagerh. sp. nov. (on Carduaceae).

Eupatorium Aschenbornianum Schauer, Cerro Quemado, Dept. Quezaltenango, Jan. 21, 1915, *100*; Zunil, Dept. Quezaltenango, Jan. 28, 1917, 0, III, *792*.

Eupatorium sp., San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 7, 1915, *16*.

A specimen was distributed by G. von Lagerheim bearing the name here given. It was collected on *Eupatorium* sp., at Tichincha, Ecuador, June, 1892. The species appears not to have been published. The telia are hypophyllous, the teliospores angularly oblong, 15–20 by 42–60 μ, with nearly or quite colorless walls, 1.5–2 μ thick. Although the type material from South America does not appear to show pycnia, yet they are well developed on Professor Holway's no. 792. They are epiphyllous, honey-yellow, prominent, subepidermal, globoid, 90–112 μ in diameter, with ostiolar filaments present.

63. Pucciniosira Brickelliae Diet. & Holw. (on Carduaceae).

Brickellia adenocarpa Robins., Solola, Jan. 29, 1915, *151*; Guatemala City, Feb. 8, 1916, *466*.

Brickellia adenocarpa glandulipes Robins., Quezaltenango, Jan. 20, 1915, *92*; Huehuetenango, Jan. 21, 1917, *755*; Zunil, Dept. Quezaltenango, Jan. 20, 1917, *783*.

A short-cycle rust, heretofore known only from Mexico, and one collection by Kellerman on *B. Cavanillesii* Gray, from Volcan de Cerro Quemada, Feb. 8, 1906, *5448*, and reported by Kern in Journ. Myc. *l. c.*

64. Endophyllum circumscriptum (Schw.) Whetzel & Olive (on Vitaceae).

Cissus sp., Quirigua, Dept. Zacapa, March 22, 1916, *506*; San Felipe, Dept. Retalhuleu, Jan. 12, 1917, *695*; same, Jan. 14, 1917, *720*.

This short-cycle rust was collected by Kellerman on *Cissus sicyoides* L., at Los Amates, Dept. Izabel, Jan. 17, 1905, *5335*, and at Gualan, Dept. Zacapa, Dec. 28, 1905, *5440*, and reported by Kern in Journ. Myc. *l. c.* It is rather common in the West Indies and South America.

65. Endophyllum decoloratum (Schw.) Whetzel & Olive (on Carduaceae).

Clibadium Donnell-Smithii Coult.

A collection of this short-cycle rust was seen in the cryptogamic

herbarium of the New York Botanical Garden, under the synonymous name *Aecidium Clibadii* Syd., made at Guatemala City, February, 1890, by J. Donnell Smith. The species is also known from Mexico, Porto Rico, and from South America.

66. ENDOPHYLLOIDES PORTORICENSIS Whetzel & Olive (on Carduaceae).

Mikania cordifolia (L.f.) Willd., Retalhuleu, Feb. 26, 1916, *538*.

Mikania sp., Puerto Barrios, March 26, 1916, *603*.

This short-cycle species appears to be most abundant in Porto Rico, but was found in the phanerogamic herbarium at the New York Botanical Garden, on *M. scandens* Willd., from Aspinwall, Panama, Hayes, *868*, and on the same host from the vicinity of Secanquim, Dept. Alta Vera Paz, Guatemala, Jan. 11, 1905, Maxon and Hay, *3239*.

67. UROMYCES CLIGNYI Pat. & Hariot (on Poaceae).

Andropogon hirtiflorus (Nees) Kunth (host det. by Hitchcock), San Rafael, Dept. Guatemala, 7000 feet alt., Jan. 10, 1915, II, III, *57*; Solola, 7000 feet alt., Jan. 25, 1915, 1915, II, III, *114*.

A rather abundant, heteroecious species in Mexico, also found in tropical Africa. Aecia are unknown.

68. UROMYCES LEPTODERMUS Sydow (on Poaceae).

Panicum barbinode Trin., Guatemala City, 4800 feet alt., Jan. 2, 1915, II, *12*.

This common tropical rust was also collected by Kellerman, on *Panicum barbinode* Trin., at Laguna, Dept. Amatitlan, Feb. 5, 1905, II, *5364*, and reported by Kern in Mycologia *l. c.*, and on *P. Liebmannianum* Trin., at Guatemala City, Feb. 2, 1905, II, *5376*.

The species ranges from Florida into South America, being very common in the West Indies. It also occurs in India. The alternate stage is unknown.

69. UROMYCES ERAGROSTIDIS Tracy (on Poaceae).

Eragrostis limbata Fourn., Solola, 7000 feet alt., Jan. 31, 1915, II, III, *167*; Antigua, Dept. Sacatépequez, Dec. 28, 1916, ii, III, *651*.

The species is common in the southern United States and Mexico, and apparently local in the West Indies. The aecial stage is not known.

70. UROMYCES EPICAMPUS Diet. & Holw. (on Poaceae).

Epicampes macroura Benth., San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 7, 1915, II, III, *26*.

Known also from Mexico, but a rather rare species. The aecia are as yet unknown.

71. UROMYCES COMMELINAE (Speg.) Cooke (on Commelinaceae).

Tradescantia cumanensis Kunth, Guatemala City, Jan. 9, 1917, II, *683*; San Felipe, Dept. Retalhuleu, Jan. 13, 1917, II, *710*.

This imperfectly known rust rarely produces telia in the warmer regions of its range. This is the only record for its occurrence on Tradescantia, except the type collection from Argentina.

72. **Uromyces socius** Arth. & Holw. sp. nov. (on Loranthaceae).

Loranthus crassipes Oliver (?), Solola, 6000 feet alt., Feb. 1, 1915, I_2, III, *169*; San Lucas Toliman, 5100 feet alt., Dept. Solola, Feb. 3, 1915, I_2, III, *185*.

Loranthus sp., Antigua, Dept. Sacatépequez, March 1, 1916, II, III, *539*; same, March 2, 1916, I_2, III, *545* (type); Panajachel, Dept. Solola, Jan. 3, 1917, III, *665*.

Struthanthus densiflorus (Benth.) Mart., Huehuetenango, Jan. 22, 1917, ii, III, *765*.

Aecia chiefly hypophyllous, crowded upon distended bladder-like areas 0.3–2 cm. across, short cylindric, 0.4–0.8 mm. in diameter, about 0.4 mm. high; peridium erect, erose; peridial cells rectangular or rhombic in side view, 23–26 by 35–45 μ, abutted or slightly overlapping, the outer wall 4–8 μ thick, transversely striate, smooth, the inner wall 7–10 μ thick, closely verrucose; aeciospores ellipsoid or oblong, 23–27 by 26–35 μ; wall colorless, 2–3 μ thick, closely and finely verrucose.

Uredinia mostly hypophyllous, crowded in small circinating groups 1–4 mm. across, soon filled with teliospores, early naked, cinnamon-brown, pulverulent, ruptured epidermis evident; urediniospores fusiform or fusiform-ellipsoid, 16–26 by 37–55 μ; wall golden-brown, 1.5–2.5 μ thick, moderately echinulate, the pores distinct, 4, equatorial.

Telia amphigenous, crowded in small circinating groups 1–4 mm. across, ellipsoid or oblong, 0.3–0.8 mm. long, compact, blackish, ruptured epidermis conspicuous; teliospores ellipsoid, obovoid or oblong-obovoid, 18–24 by 29–35 μ, rounded at both ends or somewhat narrowed below; wall dark chestnut-brown, 2–3 μ thick, thicker above, 5–9 μ, longitudinally verrucose-rugose in more or less broken lines 1–3 μ apart; pedicel colorless, as long as the spore, verrucosely roughened.

The uredinia of this species are sparingly formed, although urediniospores are abundant, being produced along with the teliospores. The aecia are unaccompanied by pycnia and seem to be secondary aecia. In these respects the species is like *U. ornatipes* Arth. on *Loranthus Sonorae*. In *U. circumscriptus* Neger and *U. Urbanianus* P. Henn., both on Loranthaceae from South America, no urediniospores have been recorded. This rust differs materially from *U. ornatipes* by absence of transverse wrinkling in the telial pedicels and by larger and differently shaped urediniospores. It differs from *U. circumscriptus* and *U. Urbanianus* by the rugose sculpturing of the teliospores in addtion to the verrucose markings, as well as in the presence of uredinia.

73. UROMYCES IRESINES Lagerh. (on Amaranthaceae).

Iresine Celosia L. (*I. celosioides* L.), Solola, Jan. 28, 1915, I, III, *141*; Aguas Amargas, Dept. Quezaltenango, Jan. 30, 1917, I, III, *803*.

A description of this species was first published by Sydow in his Monog. Ured. **2**: 227. 1910, from material collected by Lagerheim in Ecuador, on an undetermined Iresine, only teliospores being seen. Mention was made of its resemblance to a leptoform. The telia are very pale, almost colorless, and the spores germinate freely in the sorus. Ferdinandsen and Winge in their account of the fungi of the Virgin Islands, then the Danish West Indies (Bot. Tidskr. **29**: 8. 1908), speak of "unripe" teliospores, in connection with *Puccinia macropoda* Speg. on *Iresine elatior*, which upon examination prove to be this species. The specimen came from the island of St. Thomas, and shows only telia.

The present material shows an especially fine development of the species. In both gatherings there are aecia, at first appearing in epiphyllous groups, which later become surrounded by telia on either or on both surfaces. Telia also occur independent of aecia. Where both forms occur together they are on pale spots, 2–6 mm. across, and are so unmistakably from the same mycelium that in spite of the leptoform of the telia they must be considered stages of one and the same species. No pycnia could be detected, even in the youngest stages of development. The aecia may be described as follows:

Aecia epiphyllous, gregarious on pale spots, 2–4 mm. across, round, about 0.2 mm. across, opening by a pore, in cross section definitely globoid, 190–220 μ in diameter, surrounded and overarched by the host tissue; peridium wanting; aeciospores irregularly ellipsoid,

18–21 by 25–31 μ; wall pale or nearly colorless, thin, 1 μ, moderately verrucose with distinct, rather blunt warts.

74. UROMYCES CELOSIAE Diet. & Holw. (on Amaranthaceae).

Iresine Calea (Ib.) Standley (*I. latifolia* Benth. & Hook.), Antigua, 5300 feet alt., Dept. Sacatépequez, Jan. 10, 1915, II, III, *76*; Solola, Jan. 27, 1915, II, III, *128*.

The species was collected by Kellerman on *I. Calea*, at Guatemala City, Feb. 2, 1905, II, III, *4344*, *5379*; Laguna, Lake Amatitlan, Feb. 5, 1905, II, III, *5371*, and Jan. 20, 1906, II, III, *5395*; Antigua, Dept. Sacatépequez, Feb. 13, 1905, II, *5339*; except nos. *4344* and *5395* these were reported by Kern in Journ. Myc. *l. c.* The host for all the Holway and Kellerman numbers here cited were redetermined, July 3, 1917, by Paul C. Standley, who recently monographed the genus for the North American Flora. Aecia have not yet been found.

75. UROMYCES APPENDICULATUS (Pers.) Fries (on Fabaceae).

Phaseolus atropurpureus DC., Laguna, Lake Amatitlan, Feb. 8, 1915, II, *202*.

Phaseolus lunatus L., Panajachel, Dept. Solola, Jan. 30, 1915, II, III, *159*.

Phaseolus sp., Antigua, Dept. Sacatépequez, Jan. 11, 1915, II, *67*; Quezaltenango, Jan. 21, 1915, III, *105*; Solola, Jan. 25, 1915, ii, III, *117*; Moran, Dept. Amatitlan, Dec. 22, 1916, II, III, *620*.

This common autoecious rust was also collected by Kellerman on *P. atropurpureus*, at Laguna, Feb. 5, 1905, II, III, *5372*, and reported by Kern in Mycologia *l. c.* It is not a common species in warm regions.

76. UROMYCES FABAE (Pers.) DeB. (on Fabaceae).

Faba vulgaris L., Aguas Amargas, Dept. Quezaltenango, Jan. 30, 1917, II, *796*.

A common autoecious species northward but rare in warmer regions.

77. UROMYCES PUNCTATUS Schröt. (on Fabaceae).

Astragalus guatemalensis Hemsl., Quezaltenango, Jan. 16, 1917, II, III, *736*.

A common species in tropical regions. It is considered heteroecious, with aecia on Euphorbia. The aecia have not been found in America.

78. UROMYCES HEDYSARI-PANICULATI (Schw.) Farl. (on Fabaceae).
Meibomia angustifolia (H.B.K.) Kuntze, Guatemala City, March 17, 1916, II, III, *589*.
Meibomia scorpiurus (L.) Kuntze, Mazatenango, Feb. 25, 1916, II, *528*.
Meibomia tortuosa (Sw.) DC., Solola, Jan. 31, 1915, II, III, *163*; Antigua, Dept. Sacatépequez, Dec. 28, 1916, II, III, *646*.
Meibomia sp., Antigua, Dept. Sacatépequez, March 9, 1916, II, III, *583*; Huehuetenango, Jan. 22, 1917, II, III, *761*; same, II, *764*.

The host of nos. 761 and 764 is a shrubby species. The rust is notable in possessing more strongly developed paraphyses than any collection before seen, being incurved and considerably thickened along the convex wall. The aecia of this long-cycle rust are rarely collected. Uredinia of the species were detected on *M. scorpiurus* in the herbarium of the New York Botanical Garden, on a phanerogamic specimen collected by Maxon and Hay, at Las Animas near Mazatenango, Feb. 16, 1905, *3450*.

79. UROMYCES TRIFOLII (Hedw.f.) Lev. (on Fabaceae).
Trifolium amabile H.B.K., San Rafael, Dept. Guatemala, Jan. 9, 1915, II, III, *48*.

A common autoecious species in temperate regions.

80. UROMYCES COLOGANIAE Arth. (on Fabaceae).
Cologania glabrior Rose, San Rafael, Dept. Guatemala, Jan. 7, 1915, II, *31*; Guatemala City, Dec. 20, 1916, II, *609*.

A long-cycle species, whose aecia are unknown. It occurs also in Mexico and Porto Rico.

81. UROMYCES GUATEMALENSIS Vestergr. (on Fabaceae).
Bauhinia inermis Pers., Patulul, 600 feet alt., Dept. Escuintla, Feb. 4, 1915, ii, III, *194*.

A long-cycle rust, whose initial stage is unknown. It was first detected on a phanerogamic specimen collected by Bermoulli and Cario, on an undetermined species of Bauhinia, at Retalhuleu, March, 1876, *1311*, and made the type of the species by Vestergren in Arkiv för Botanik.

82. UROMYCES MONTANUS Arth. (on Fabaceae).
Lupinus montanus H.B.K., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, 0, I, III, *576*.

When this species was published in 1905 from Mexican material, it was considered that distinctly leptoform telia, with an abundant germination of teliospores taking place as rapidly as they matured, were incompatible with the association of grouped aecia accompanied by pycnia, such telia having always been considered short-cycled. The present collection shows the same intimate association of aecia and germinating telia, as also does a collection by Kellerman, Feb. 5, 1908, from the same region, on an undetermined Lupinus.

A similar association is also to be seen in *Uromyces elatus* Syd., from South America. In that species the telia are not dark brown, but very pale brown, the spores being almost colorless under the microscope. The teliospores of the South American species do not germinate so readily, but they are of the leptosporic form, having thin and delicate walls. No telia of either species have so far been found, except those with the aecia closely associated, although some collections of aecia of both sorts have been found without telia. Both species are high-altitude forms.

In view of the constant association of aecia and telia, both in this species and in *U. elatus*, it is now reluctantly admitted that there is strong likelihood of genetic connection. The aecia found with telia of *U. montanus*, and those of like characters but not so associated, are here placed under the name *U. montanus*. Such aecia have heretofore been placed with *U. Lupini* B. & C. Final decision must depend upon cultures.

It is found by further study with more abundant material, that the aecia of *U. Lupini* are somewhat smaller and thinner-walled than those of the other two species mentioned. The aecia of *U. montanus* and *U. elatus* have each a distinctive macroscopic appearance, the former being cupulate and in circinating groups, while the latter are cylindric (not so stated in the original description, as the type specimens were immature) and in small groups of a few sori each, giving the appearance of irregular distribution.

83. **Uromyces illotus** Arth. & Hol. sp. nov. (on Fabaceae).

Mucuna Andreana Micheli, Chinaulta, Dept. Guatemala, Feb. 12, 1916, II, iii, *487*.

Uredinia hypophyllous, scattered, round or oval, 0.2–0.5 mm. across, early naked, pulverulent, dark cinnamon-brown, ruptured epidermis inconspicuous; urediniospores obovoid, 16–23 by 24–27 μ; wall cinnamon-brown, moderately thick, 1.5 μ, moderately echinulate, the pores 3–4, equatorial or sometimes scattered.

Telia hypophyllous, scattered, round, 0.1–0.3 mm. across, early naked, compact, dark chocolate-brown, ruptured epidermis inconspicuous; teliospores obovoid or ellipsoid, 19–21 by 24–31 μ, rounded at both ends; wall dark chestnut-brown, thick, 2–2.5 μ, thicker above up to 5 μ, closely and finely verrucose; pedicel colorless, once to once and a half length of spore.

This rust differs from *Uromyces Mucunae* Rab. in its larger urediniospores and teliospores, and in the absence of uredinial paraphyses. Part of the original collection of *Uredo mucunicola* P. Henn. in the possession of the writer has yielded a few teliospores, which show it to be identical with *Uromyces Mucunae* Rab., for which it should be entered as a synonym.

84. UROMYCES INDIGOFERAE Diet. & Holw. (on Fabaceae).

Indigofera mucronata Spreng.

The species was collected by Kellerman, at Gualan, Dept. Zacapa, Dec. 28, 1905, II, *5444*, and reported by Kern in Journ. Myc. *l. c.* The species is known from central Texas, southward through Mexico, but is not often collected.

85. UROMYCES PROEMINENS (DC.) Pass. (on Euphorbiaceae).

Chamaesyce brasiliensis (Lam.) Small (*Euphorbia brasiliensis* Lam.), Retalhuleu, Feb. 26, 1916, II, III, *533*.

Chamaesyce hirta (L.) Millsp. (*Euphorbia hirta* L.), Solola, Jan. 28, 1915, I, ii, *142*; Sanarate, Dept. Guatemala, Feb. 10, 1916, I, II, *477*.

The species is a common long-cycle form. It was collected by Kellerman on *C. adenoptera* (Bertol) Small, at Los Amates, Dept. Izabel, Jan. 5, 1908, II, III, *7036*, and on *C. lasiocarpa* (Klotsch) Arth., Laguna, Lake Amatitlan, Jan. 17, 1906, II, III, *5404*, both reported by Kern in Mycologia *l. c.*, and on *C. brasiliensis*, at Laguna, Lake Amatitlan, Feb. 8, 1905, II, III, *5341*.

The species was also detected in the Field Museum, on a phanerogamic specimen of *Eumecanthus gramineus* (Jacq.) Millsp. (*Euphorbia graminea* Jacq.), sheet no. 247010, collected at Agua Caliente, June 2, 1909, by C. C. Deam, *6137*. Only uredinia were present.

86. UROMYCES OAXACANUS Diet. & Holw. (on Euphorbiaceae).

Jatropha urens L., Guatemala City, Dec. 31, 1914, II, iii, *2*.

A species heretofore known only from southern Mexico has not been found showing pycnia. It is doubtful if aecia occur.

87. UROMYCES GOUANIAE Kern (on Frangulaceae).
Gouania lupuloides (L.) Urban (*G. domingensis* L.).
Only the type collection of this species is known, which was obtained by Kellerman at Laguna, Lake Amatitlan, Jan. 25, 1906, II, III, *5391*. It was described in Mycologia (3: 290. 1911).

88. UROMYCES HYPERICI-FRONDOSI (Schw.) Arth. (on Hypericaceae).
Hypericum pratense Schl. & Cham., San Rafael, Dept. Guatemala, Jan. 7, 1915, III, *25*.
A long-cycle, autoecious rust, found in Mexico and northward, whose aecia are not uncommon in the cooler regions.

89. UROMYCES HOWEI Peck (on Asclepiadaceae).
Asclepias curassavica L., Laguna, Lake Amatitlan, Feb. 8, 1915, II, *203*.
Asclepias guatemalensis Donn. Sm., San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 7, 1915, II, III, *20*.
A common rust in Canada and the United States east of the Rocky Mountains, and much less so in Mexico and the West Indies. The beginning stages of the life cycle are unknown.

90. UROMYCES CESTRI Mont. (on Solanaceae).
Cestrum aurantiacum Lindl., Solola, 7000 feet alt., Jan. 28, 1915, I, III, *143*.
A South American rust, common in the West Indies where the aecia produce discoid galls or hypertrophy. It has not before been reported from the continent of North America.

91. UROMYCES MACULANS (Pat.) Arth. (on Solanaceae).
Cestrum lanatum Mart. & Gal., Chinautla, Dept. Guatemala, Feb. 12, 1916, I, *489*.
The species also occurs in Costa Rica on *C. nocturnum* L. It is a long-cycle rust with aecia and telia, but no uredinia.

92. UROMYCES SOLANI Diet. & Holw. (on Solanaceae).
Solanum nudum H.B.K., Quezaltenango, Jan. 31, 1917, II, *815*.
An imperfectly known rust, pycnia and aecia yet undiscovered, heretofore known from two localities in southern Mexico.

93. UROMYCES BOUVARDIAE Sydow (on Rubiaceae).
Bouvardia leiantha Benth., Guatemala City, Dec. 20, 1916, II, III, 605.
This collection tallies closely with the type collection of the species,

also from Guatemala, in size of spores and in having pedicels that are not inflated. Most collections from Mexico possess strongly inflated pedicels, with thicker walls to the urediniospores and teliospores, larger spores, and other correlated differences. There are, however, collections showing intermediate characters, as one from Chapala, Mex. (Barth. N. Am. Ured. *186*), which also has pycnia and aecia present. Although the variation is considerable, yet there does not seem to be sufficient reason at present to consider the Mexican form distinct from the Guatemalan one.

The type collection was made by Heyde and Lux at Jumaytepeque, Dept. Santa Rosa (Ann. Myc. 1: 16. 1903), and on *Bouvardia leiantha.*

94. UROMYCES HELLERIANUS Arth. (on Cucurbitaceae).

Melothria scabra Naud., Guatemala City, Dec. 23, 1916, II, III, *630.*

Melothria sp., Chinautla, Dept. Guatemala, Feb. 12, 1916, II, III, *483.*

Genus and species undetermined, Mendez, Dept. Guatemala, Feb. 13, 1917, ii, III, *862.*

The early stages of this long-cycle rust have not yet been discovered. The species was collected by Kellerman on *Cayaponia racemosa scaberrima* Cogn., at Moran, Dept. Amatitlan, February 1906, ii, III, *5436*, and reported by Kern in Journ. Myc. *l. c.* The three collections here listed are the only ones known except from the West Indies.

95. UROMYCES PRESSUS Arth. & Holw. (on Carduaceae).

Vernonia Deppeana Less., San Lucas Toliman, 5100 feet alt., Dept. Solola, Feb. 2, 1915, II, *173*; Malacatancito, Dept. Huehuetenango, Jan. 24, 1917, II, *779.*

The life cycle includes pycnia, uredinia, and telia. The species also occurs in Costa Rica on the same host.

96. UROMYCES POLYMNIAE (P. Henn.) Diet. & Holw. (on Carduaceae).

Polymnia maculata Cav., San Rafael, Dept. Guatemala, Jan. 7, 1915, II, iii, *30*; same, 7000 feet alt., Jan. 10, 1915, II, *62*; Volcan de Agua, Dept. Sacatépequez, March 4, 1916, III, *553*; Quezaltenango, Jan. 18, 1917, II, III, *749.*

A long-cycle rust, having pycnia, aecia, uredinia, and telia, common in Mexico, and also occurring in South America.

97. UROMYCES CUCULLATUS Sydow (on Carduaceae).

Perymenium strigillosum (Rob. & Greenm.) Antigua, Dept. Sacaté-

pequez, March 1, 1916, ii, III, *541*; Guatemala City, Jan. 7, 1917, II, III, *679*.

Perymenium Purpusii Brandegee, Quezaltenango, Jan. 16, 1916, II, III, *734*.

Zexmenia scandens Hemsl., San Rafael, Dept. Guatemala, Jan. 9, 1915, ii, III, *43*; San Lucas Toliman, Dept. Solola, Feb. 3, 1915, ii, III, *183*; Panajachel, Dept. Solola, Jan. 3, 1917, II, III, *668*.

A long-cycle species with aecia, very common in Mexico.

98. **Uromyces Salmeae** Arth. & Hol. sp. nov. (on Carduaceae).

Salmea scandens (L). DC., San Lucas Toliman, 7000 feet alt., Dept. Solola, Feb. 3, 1915, 0, I, II, iii, *188*.

Pycnia amphigenous, few in groups on discolored spots, subepidermal, noticeable, globoid or ellipsoid, 160–200 μ broad.

Aecia amphigenous, grouped, cupulate, 0.1–0.2 mm. in diameter; peridium delicate, short; peridial cells soon collapsing, thin-walled, 1 μ, coarsely and closely verrucose; aeciospores ellipsoid or oblong, 19–25 by 24–35 μ, wall light cinnamon-brown, 1.5–2 μ, usually thicker above, up to 5 μ, closely and coarsely verrucose.

Uredinia mostly hypophyllous, scattered, oval or oblong, 0.3–0.8 mm. long, early naked, pulverulent, ruptured epidermis evident; urediniospores obovoid or ellipsoid, 23–27 by 30–35 μ; wall cinnamon-brown, 1–1.5 μ, moderately echinulate, the pores 2, slightly supra-equatorial.

Telia hypophyllous, scattered, oval or oblong, 0.3–0.8 mm. long, early naked, somewhat pulverulent, light chestnut-brown, ruptured epidermis noticeable; teliospores oblong or narrowly ellipsoid, 18–23 by 35–50 μ, narrowed above and below; wall golden-brown above, lighter to colorless below, thin, 1 μ, much thickened above, 5–13 μ, smooth; pedicel colorless, fragile, as long as the spore.

99. Uromyces columbianus Mayor (on Carduaceae).

Melanthera aspera (Jacq.) Steud., Escuintla, Feb. 18, 1916, II. *507*; Mazatenango, Feb. 22, 1916, II, *512*; San Felipe, Dept. Retalhuleu, Jan. 13, 1917, 0, I, II, iii, *711*.

Melanthera oxylepis DC., Panajachel, Dept. Solola, Jan. 3, 1917, 0, I, II, *672*.

Melanthera sp., Quirigua, Dept. Zacapa, March 22, 1916, I, II, *602*.

A long-cycle rust with all spore forms, common in Central America and the West Indies, as well as in South America.

100. UROMYCES BIDENTICOLA (P. Henn.) Arth. (on Carduaceae).

Bidens heterophylla Ort., Quezaltenango, Jan. 18, 1917, II, *747*.

Bidens Holwayi Sherff & Blake, Quezaltenango, Jan. 31, 1917, 0, II_1, II_2, iii, *816*.

Bidens pilosa L., San Rafael, Dept. Guatemala, Jan. 7, 1915, II, *27*.

Bidens squarrosa H.B.K. (*B. tereticaulis* DC.), Guatemala City, Dec. 31, 1914, II, III, *4*; Solola, 7000 feet alt., Jan. 25, 1915, II, III, *110*; Zunil, Dept. Quezaltenango, Jan. 28, 1917, 0, II, *786*.

A long-cycle rust, having pycnia, uredinia, and telia, common in the American tropics, especially in the uredinial stage. It has generally been listed as *U. Bidentis* Lagerh., a name that properly belongs to the similar short-cycle form. The rust in the uredinial stage was collected at Amatitlan, on *B. pilosa*, date not given, by Heyde and Lux, and on *B. leucantha* Willd., in January, 1876, place not given, by Bernoulli and Cario.

101. UROMYCES MONTANOAE Arth. & Holw. (on Carduaceae).

Montanoa hibiscifolia Benth., San Felipe, Dept. Retalhuleu, Jan. 13, 1917, II, *705*.

Montanoa Pittieri Rob. & Greenm., Antigua, 5300 feet alt., Dept. Sacatépequez, Jan. 12, 1915, II, *77*; San Lucas Toliman, 5100 feet alt., Dept. Solola, Feb. 2, 1915, II, III, *176*; Moran, Dept. Amatitlan, Dec. 22, II, III, *625*.

A rust similar to *Uromyces bidenticola*, but distinguishable in the urediniospores. As in that species, some of the telia show early germination in evident association with the uredinia. The genus Montanoa is somewhat but not closely related to Bidens.

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CALCIUM OXALATE IN THE DASHEEN*

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Calcium oxalate enters into the composition of a great variety of plants. As it is a quite insoluble salt, when synthesized by the plant it separates as a solid from the plant juices. It is probably formed during protein metabolism and is considered by most authorities as a waste product of such action, although a few plant physiologists maintain that it is dissolved and utilized in the further growth of the plant. However, the solid crystals may easily be detected under the microscope in various forms in plant tissues. Not infrequently they are found in bundles of fine, needle-like crystals packed in cells and surrounded by a mucilaginous liquid. Crystals of this character are called "raphides," and the cells containing them when brought in contact with water have the property of ejecting the individual needles which float out slowly into the surrounding medium. Plants which produce calcium oxalate in this form, when eaten raw, invariably cause a painful burning sensation in the mouth, the obvious explanation of which is that when the cells full of needles meet the saliva of the mouth the needles are ejected and penetrate the mucous membrane, although other explanations are conceivable.

Among the plants which synthesize calcium oxalate in this interesting form is the dasheen (*Colocasia esculenta* (L.) Schott.), which has been introduced into this country by the Office of Seed and Plant Introduction of the United States Department of Agriculture, and has been successfully grown in Florida, where it has shown every promise of becoming a valuable addition to our vegetable food supply. The tubers of this plant can be utilized as a substitute for potatoes, while the large leaves when boiled have proved excellent greens. There

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has, nevertheless, been a distinct prejudice against the plant which has acted adversely to its extended use, due to the intensely acrid taste of the raw leaves and to a less degree of the tubers. Both portions of the plant were known to possess the raphide-filled cells, and this has been assumed to be the cause of their acridity. There were, however, certain reasons for suspecting that this was not entirely the case, and as the plant is one of considerable food value it seemed advisable to make a study of it in this respect.

The evidence that raphides are calcium oxalate and not calcium citrate is very complete, although some confusion has existed on this point, as the two salts have many properties in common. By careful analytical work F. G. Kohl[1] has shown beyond reasonable doubt that raphides are the oxalate, and quite recently H. Ziegenspeck,[2] who seems to be unfamiliar with Kohl's research, has prepared from raphides pure oxalic acid which he identified by its characteristic physical properties, thus furnishing absolutely conclusive proof of the composition of the crystals.

On the question of the cause of the acrid taste of raphide-containing plants the evidence is less direct. Much has been written about such plants, the general opinion seeming to be that the raphides serve as a protection to the plants against animals and insects, although one writer, A. Schneider,[3] takes the ground that such is not the case, but, on the contrary, that calcium oxalate crystals serve the plants as a tissue support and add to their structural rigidity. E. Stahl[4] studied the effect of a variety of raphide-bearing plants when fed to hungry snails and reached the conclusion that the burning taste depends on the raphides alone, but his conclusion is arrived at through a process of reasoning rather than by experiment. The experiment of Barnes[5] has a more direct bearing on the question. He macerated a raphide-bearing plant in water and then subjected it to filtration through such a fine filtering medium that no raphides could pass. The resulting filtrate showed no sign of acridity, and hence he concluded that the raphides were solely responsible by mechanically irritating the mouth. As raphides were not the only material removed by the filtration, his conclusion was rather more sweeping than the experiment warranted.

[1] Anatomisch-physiologische Untersuchungen der Kalksalze und der Kieselsäure in der Pflanze. p. 91. Marburg, 1889.

[2] Ber. Deutsch. Bot. Ges. 32: 630–633. 1915.

[3] Bot. Gaz. 32: 142–144. 1901.

[4] Pflanzen und Schnecken. Jena, 1886.

[5] Barnes, C. R. Bot. Gaz. 13: 232–233. 1888.

Samples of dasheen, both tubers and leaves, were placed at the writer's disposal through the kindness of Mr. R. A. Young, of the Office of Foreign Seed and Plant Introduction, and they were used in the work which follows in an endeavor to settle the point as to whether the raphide content was the sole factor in causing the acridity of this plant.

The family of the Araceae to which the dasheen belongs is known to produce in several instances alkaloids, glucosides, and bitter substances, any of which if present in the plant under investigation might be wholly or partially responsible for its peculiar flavor. Samples of dasheen leaves were, therefore, distilled with steam on the assumption that they might contain a volatile compound, possibly an alkaloid. The distillate, however, failed to show the presence of any body of this nature. Further experiments were made on the dried leaves, which were exhaustively extracted with various solvents, namely, petroleum ether, ethyl ether, chloroform, acetic ether, alcohol, and water in rotation. These extracts, likewise, after evaporating the solvent, showed no evidence of an acrid flavor, but the observation was made that the residual leaf matter was no longer unpleasant to the taste, although when examined under the microscope the raphide cells were still intact and apparently in perfect condition. At first sight this seemed to be proof that the raphides had no connection with the acrid taste of the leaves, but on further investigation it was found that when cells from these extracted leaves were brought into contact with water they had wholly lost their capacity to expel the individual needle crystals, a fact which might very well acount for their loss of virulence. The facts brought out in this series of experiments, namely, that the various extracts and the residue were all without acrid effect, seem to force us back on the raphide theory of acridity, with the additional proviso that free movement of the raphides is necessary to secure the effect. It might, nevertheless, be contended that acridity is destroyed in the process of extraction by the decomposition of some compound, though the possibility seems remote.

To secure more direct evidence of the mechanical effect of raphides on the mouth an attempt was made, with a moderate degree of success, to synthesize calcium oxalate in fine needle forms as near as could be in size and shape to naturally-occurring raphides. It was found that when a dilute solution of calcium chloride was slowly dropped into a dilute solution of oxalic acid, crystals of calcium oxalate separated,

which were of sharp monoclinic form and largely admixed with needle-like crystals quite similar to natural raphides and of approximately the same size. To observe the effect of this preparation on the mouth, the crystals were filtered, washed with water, dried, and then incorporated in melted paraffin. When the gum thus produced was masticated for a short while it left in the mouth a burning sensation comparable to that experienced from dasheen leaves, though less intense.

An endeavor to isolate the naturally-formed raphides was only partially successful, but the attempt served to bring out further evidence that the raphides are responsible for the acrid taste. Corms of the Indian turnip (*Arisaema triphyllum* (L.) Torr.) were used in this experiment, since they are easily obtained in the vicinity of Washington, contain raphides in large quantities, and have an intensely acrid flavor. The turnip-like corms were peeled, washed, and grated to a pulp which was supended in distilled water for some time to allow the needle crystals to work out of the cells. The mixture was then filtered by suction through a rather coarse cloth, stirred up again with water and again filtered. A drop of filtrate, under the microscope, showed the presence of many needle crystals mixed with some starch granules. The filtrate was next centrifuged, the supernatant liquid poured off, the residue stirred up with distilled water and again centrifuged, this process being carried through several times until the mixed raphides and starch had been well washed. The solid residue from these operations had an intensely acrid flavor, differing in no respect from that of the untreated tuber.

An endeavor was made to obtain the raphides in a still purer form by eliminating the starch through the action of diastase. The washed mixture of starch and raphides was subjected to the action of yeast over night at a temperature of 40° C. When the preparation was examined the following morning, however, no raphides were found in it, and, moreover, it had lost entirely its acrid taste. This experiment, therefore, while it failed of its original object, nevertheless offers strong additional proof that raphides are the cause of acridity.

In repeating the above described experiment on the dasheen, similar observations were made.

Conclusions

All experimental evidence goes to show that calcium oxalate crystals are the sole cause of the acrid taste of the dasheen by the mechanical irritation of the mucous membrane of the mouth.

As the acrid flavor can readily be removed by proper methods of cooking,[6] there is no reason why the presence of raphides should interfere with the use of the plant as a vegetable.

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[6] Young, R. A. Yearbook U. S. Dept. Agric. 1916. pp. 203–206.

THE INFLUENCE OF CERTAIN ADDED SOLIDS UPON THE COMPOSITION AND EFFICIENCY OF KNOP'S NUTRIENT SOLUTION

E. H. Toole and W. E. Tottingham

It was pointed out many years ago by Nägeli (1) that such insoluble substances as filter paper and paraffin shavings have the power of rendering water from a copper still suitable for the culture of algae. This observation led True and Oglevee (2) to make a study of the effect of these and other similar substances on the growth of plants in various solutions, the toxicity of which had previously been determined. They found that solutions of copper sulfate, silver nitrate, and other substances, in concentrations near their toxic limits, were rendered markedly less toxic or even stimulating to lupine radicles when accompanied by such substances as shredded filter paper, freshly prepared potato starch and fine sea sand in amounts equal to about one third that of the culture solution. These authors comment on the adsorbing power of soils and its relation to plant growth.

Breazeale (3) studied the question from another angle. To soil extracts which supported but a poor growth of seedlings were added such substances as washed carbon black, calcium carbonate, ferric hydrate, aluminum hydrate, and quartz flour. Ferric hydrate was especially effective in causing an increased development of the roots of wheat seedlings. It was also found that these solids have a remarkable power of suppressing the toxic properties of distilled water. Parker (4) has measured the power of soils to adsorb salts from solutions and found not only that the concentrations of salts may be changed, but also that there is selective adsorption of certain ions from the solution.

Consideration of these and related observations led the writers to investigate the effects upon the physiological balance of a nutrient solution, of various added solids supposed to offer relatively large presentations of surface. It was deemed possible that, if changes in the solution occurred under these conditions, the evidence obtained might render possible a distinction between adsorptive effects and ordinary chemical reactions, as causal agents. Rather unsuspected

relations have been disclosed by the results obtained, which seem to justify their publication as matters of general interest.

Methods of Investigation

Young seedlings of Chevalier barley and Little Gem peas were grown, according to the method previously described by Tottingham (5), in pint jars containg Knop's solution[1] of about 0.3 percent total concentration in which had been incorporated a definite amount of a watery suspension of the solid to be studied. Six plants were grown in each jar, and each modification of the solution was employed in duplicate. The substances added to the usual nutrient solution were, respectively: silicic acid, ferric hydrate, and carbon black.

The silicic acid was prepared by adding slowly, with constant stirring, the required amount of dilute HCl to a cold, dilute solution of sodium silicate. Preliminary tests had shown this to give the most satisfactory material. The gelatinous precipitate was washed with water, by decantation, until the clear washing fluid gave no test for chlorides. Ferric hydrate was prepared by dissolving ferric oxide in hot, dilute nitric acid. The ferric nitrate solution thus formed was diluted, precipitated cold with dilute ammonia water until neutral to litmus, and the precipitated hydrate was washed by decantation until free from nitrate. "C-Elf" brand of carbon black was washed with dilute hydrochloric acid and then repeatedly washed with hot water until the wash water no longer gave visible evidence of impurities resembling oil or tar. In each case the solid was allowed to settle over night in water; then the clear, upper layer of water was siphoned away and the actual concentration of the thoroughly agitated suspension of each substance was determined by evaporating aliquot portions. In view of the uncertainty regarding the composition of the precipitated ferric hydrate, as noted by Mendeleeff (6), the iron was determined as Fe_2O_3 by ignition, and from this the equivalent amount of $Fe(OH)_3$ was calculated. The evaporated residue from the silicic acid suspension was ignited and weighed as SiO_2. The nutrient solutions for each jar were made up as needed from stock solutions of the

[1] The composition of the solution is shown by the number of cc. of M/2 solution of each salt used per liter of solution: $Ca(NO_3)_2$, 20.9; $MgSO_4$, 7.1; K_2HPO_4, 2.5; KH_2PO_4, 2.5; KNO_3, 8.5. Equal parts of KH_2PO_4 and K_2HPO_4 were used for the purpose of approximating neutrality more closely than obtains where but one of these salts is used. Four drops of colloidal ferric phosphate per jar were added with each fresh portion of solution.

separate salts of M/2 concentration; the watery suspension was incorporated in making the solution up to volume.

Two series of cultures were run for each material. In one series, there was incorporated in each 400 cc. portion of nutrient solution the equivalent of 2.5 grams of dry substance of the solid to be added. In the other, the equivalent of 0.5 grams of dry substance was used. The relative fineness of division of the solids is indicated by the following volumes of each suspension required to supply 2.5 grams of dry substance: H_2SiO_3, 93.5 cc.; $Fe(OH)_3$, 85.6 cc.; carbon black, 18.3 cc.

Thrifty seedlings about five days old, selected to be as uniform in size and appearance as possible, were supported in jars by the method used by Tottingham (5). About two thirds of the pea cotyledons were removed to hasten the effect of the nutrient solution. The plants were grown in a greenhouse at Madison, Wis., from July 20 to August 10, 1914, the cultures being shaded from the intense sunlight of the period by a cheesecloth curtain. Daily aeration of the solutions was accomplished by means of an atomizer bulb, and the solutions were renewed every three days. At the end of the growth period of twenty-one days, the plants were removed and the residual nutrient solutions, after being restored to their original volumes, were tested for hydrogen-ion concentration. Later, fresh nutrient solutions were prepared to determine their initial acidity and to test for any possible adsorption after contact with the solids.

In analyzing the culture solutions, the suspensions were allowed to settle, the clear liquids were filtered off, and aliquot parts were taken for the various determinations of hydrogen-ion concentration, phosphorus, and calcium. The concentration of hydrogen ions was determined by the indicator method, using as guides the papers of Sorensen (7), Henderson (8), Hawk (9), and Clark (10). The buffer solutions used as standards were made by dissolving the proper amounts of mono- and di-basic sodium phosphate for alkalinity, and acetic acid and sodium acetate for acidity (8). Neutral red was found satisfactory as an indicator for the more alkaline solutions studied and methyl red for the more acid solutions. Phosphorus was determined by precipitation with molybdate reagent followed by magnesia mixture. The pyro-phosphate thus obtained was dissolved in hydrochloric acid, the solution was filtered, and the phosphoric acid was finally determined by precipitation with an excess of magnesia mixture and by ignition in the usual manner (11). Calcium was determined by

precipitation with ammonium oxalate and titration of the precipitate with potassium permanganate (12).

When the plants were removed from the culture solutions, the tops were severed from the roots, cut into small pieces, dried in watch crystals at 104° C., and weighed. The roots were measured, washed free from adhering solids, and treated in the same way as the tops.

RESULTS

At the end of one week, the barley receiving the higher concentration of ferric hydrate showed a decidedly darker color and a greater growth than any of the other cultures. At this time the foliage of the peas receiving carbon black appeared somewhat darker, but the barley similarly treated was yellow, as compared with the respective control cultures.

After twenty-one days of growth the peas were just beginning to show the influence of the nutrient solutions, despite the previous removal of a large part of the reserve material of the seed. At this time the plants receiving carbon black were beginning to turn yellow and those with ferric hydrate had assumed a very dark green color.

In the barley cultures, at the end of the experiment, there was a striking contrast in the appearance of tops between the plants treated with ferric hydrate and those that had received carbon black. In the former cultures, the leaves were noticeably broader and the stems thicker than those of the control cultures, and the plants were of a very dark green color. The carbon-treated culture had produced slender plants of a yellowish green color whose general appearance was much below that of the control plants. There was a very close similarity in appearance between the plants which had received silicic acid and those of the control cultures. Those plants to which ferric hydrate had been applied appeared to have more freely branching root systems than the others, but on the whole there was a considerable uniformity in the roots of all cultures. An extra control culture of barley in distilled water plus carbon black was healthy at the end of twenty-one days, whereas the barley in distilled water alone had died some time before; hence, this added solid could hardly have been very toxic to this plant.

As more important criteria of growth than the preceding observations, the dry weights of roots and tops were measured. These results, as well as those of the study of the changes in the solution, are presented in table 1.

TABLE I

Data of Plant Yields and Analyses of Solutions for Cultures of Twenty-one Days' Duration in Knop's Solution Variously Treated

Kind of Plant	Solid Added		Yield of Dry Tops, per Plant		Yield of Dry Roots, per Plant		Length of Fresh Roots		Hydrogen-ion Concentration				Phosphorus in Solution, per Liter		Calcium in Solution, per Liter	
									Uncropped after 4 Days		Cropped, after Last 4 Days Growth		Uncropped, after 4 Days		Uncropped, after 4 Days	
	Kind	Amt.	Separate Culture	Average	Separate Culture	Average	Per Culture	Average	Per Solution	Average	Per Culture	Average	Per Solution	Average	Per Solution	Average
		grams	milligrams	milligrams	milligrams	milligrams	millimeters	millimeters	P_H*	P_H	P_H	P_H	milligrams	milligrams	milligrams	milligrams
Barley	None		97		36		215		6.0		—		86		798	
			117	107	50	43	215	215	6.0	6.0	7.4	7.4	88	87	815	806
	$Fe(OH)_3$	0.5	137		34		240									
			149	142	37	36	260	250								
	$Fe(OH)_3$	2.5	175		43		200		7.4		—		4.0		616	
			146	162	39	41	220	210	7.4	7.4	7.5	7.5	5.0	4.5	614	615
	H_2SiO_3	0.5	73		40		165									
			75	74	44	42	191	178								
	H_2SiO_3	2.5	116		59		178		6.0		—		94		812	
			88	102	50	55	162	170	6.0	6.0	7.4	7.4	84	89	790	801
	Carbon black	0.5	86		40		186									
			59	72	29	34	154	170								
	Carbon black	2.5	61		31		159		5.7		—		82		819	
			51	56	25	28	166	162	5.7	5.7	6.9	6.9	90	86	825	822
Peas	None	..	86		31		167									
			96	91	33	32	153	160								
	$Fe(OH)_3$	0.5	99		29		166									
			109	104	29	29	166	166								
	$Fe(OH)_3$	2.5	141		38		166									
			88	115	29	33	154	160								
	H_2SiO_3	0.5	71		29		114									
			56	64	22	26	108	111								
	H_2SiO_3	2.5	96		38		135									
			116	106	48	43	139	137								
	Carbon black	0.5	88		33		158									
			64	76	36	34	146	152								
	Carbon black	2.5	104		32		140									
			115	109	40	36	140	140								

* The P_H value is the number representing the logarithm of the hydrogen-ion concentration. The actual hydrogen-ion concentration is higher as the P_H value decreases. Neutrality is expressed by a P_H value of 7.0. The limiting P_H values of this table, 5.7 and 7.5, represent hydrogen-ion concentrations of 2×10^{-6} normal and 0.32×10^{-7} normal respectively; or 2 grams and 0.032 grams of hydrogen ions per million liters respectively.

Discussion of Results

The yield in dry weight of tops is in close agreement with the general appearance of the crops. It will be noted that the yield of tops of the barley cultures with a high application of ferric hydrate exceeded the control cultures by over fifty percent. The depression with the low concentration of silicic acid, as compared with the ineffectiveness of the high concentration, with both pea and barley plants, is interesting; however, these results are based upon the observation of too few plants to warrant any conclusion on this point.

The very decided depression in the yield of tops of barley when carbon black was applied would at first suggest the presence of toxins, in spite of the repeated washings of this material. In view of the fact, however, that the barley seedlings in distilled water, to which carbon black had been added, grew for three weeks with a good development of root system, while seedlings in distilled water alone were stunted in growth after a few days, it would seem that the carbon black was not inherently toxic. The young pea seedlings with the carbon black had an appearance of increased growth as long as they were supplied with nourishment from the cotyledons, but they began to look yellow as soon as this reserve was exhausted. This late development of the unfavorable effect would seem, therefore, to indicate some complex interaction between the plant and the solution, rather than a direct toxic effect of the carbon black.

The comparative uniformity, in all cultures, of both dry weight and length of root is in contrast with the results of Breazeale (3), who found a distinct stimulus to root growth on the addition of similar solids to cultures in toxic soil extracts. It is probable that the increase in dry weight of roots from the high application of silicic acid was due largely to small particles of the gelatinous material that could not be washed off. It will be noted that the high concentration of carbon black was accompanied by a depression of both length and dry weight of barley roots. Apparently, the increased growth of tops with ferric hydrate added to the solution was not caused by an increased surface of roots available for absorption but was due to some internal change in the plant, such as a change in the usual course of metabolism of the plant cells.

There is a consistent relation of the dry weight of tops in the various cultures to the hydrogen-ion concentration of the solution,

these two values varying in opposite directions; but the variations in acidity are so small that they can hardly, in themselves, explain the differences in yield. Even the comparatively high hydrogen-ion concentration of the solution which contained carbon black was decidedly below that found by Hoagland (13) to be favorable to barley seedlings in his nutrient solutions with controlled hydrogen-ion content. He found that a hydrogen-ion concentration of 0.7×10^{-5} N. (P_H, 5.2) was not injurious, while our culture with the highest acidity was 0.2×10^{-5} N. (P_H, 5.7). Attention is called to the fact that, in all cases, the growing barley seedlings had a marked neutralizing effect on the nutrient solution, and that the solutions in which the plants had been grown were more nearly uniform in hydrogen-ion concentration than the original solutions. Hoagland (13) found that acid solutions decreased slightly in hydrogen-ion concentration, that alkaline solutions decreased markedly in hydroxyl-ion concentration, and that neutral solutions tended to remain constant during the growth of barley seedlings.

The correlation of the very small amount of soluble phosphorus in the solution containing ferric hydrate with the increased yield from that solution suggests that this paucity of soluble phosphates may be the critical factor responsible for the observed results, possibly enhancing growth by rendering the solution nearly neutral. From these results, it would seem that the nutrient solution proposed by Crone (14), using the slightly soluble ferrous and tri-calcium phosphates instead of potassium phosphate, deserves more extended trial than it appears to have received. Our results are also in accord with the observations of Truog (15) that the barley plant can get a sufficient supply of phosphorus from precipitated ferric phosphate. From the uniformity of root growth in all cultures, it would seem that we have here, not a case of the direct toxicity of the phosphate to the root, but rather a difference in the use of the materials within the plant under some difference in external conditions. Although a sufficient supply of phosphorus is necessary for the full development of plants, the presence of an excess of the phosphate ion in the culture solution would seem to be, sometimes, a limiting factor in growth. Among others, McCall (16) has also noted this effect in his sand cultures. The very dark color of the leaves of all our cultures to which ferric hydrate had been added might indicate a greater utilization of the iron under these conditions.

Lack of evidence, from the analytical data, of adsorption of nutrient elements in measurable amounts by any of the solids employed here (unless we except the small decrease of calcium in the presence of ferric hydrate) is decidedly surprising. The results of True and Oglevee (2), Breazeale (3), and Parker (4), previously mentioned, and those of McCall (16) would seem to indicate that the amounts of materials added in the present investigation should have had some detectable adsorptive effect. The last-mentioned author found that contact with even relatively coarse solids affected the physiological balance of the nutrient solution as compared with water cultures. However, the ratio of solid to solution, and the composition of both, as employed by McCall, differed from those obtaining in our work. No explanation is advanced here for the depression of yield of barley by carbon black in the apparent absence of appreciable adsorption or toxicity.

Although the results here given are based on too few data to permit of anything like conclusive deductions, some of the relations herein suggested appear to be worthy of further experimental consideration. It is suggested that such changes in the substratum as those here observed influence not merely the absorbing power of the roots but probably also the metabolism of the whole plant.

SUMMARY

1. Barley and pea plants were grown twenty-one days in a Knop's nutrient solution to which had been added: (*a*) $Fe(OH)_3$; (*b*) H_2SiO_3; (*c*) carbon black; each upon two planes of application.

2. The weight of the dry barley tops was increased approximately 50 percent by the addition of $Fe(OH)_3$ to the solution, was appreciably depressed by the addition of carbon black, and was unaffected by H_2SiO_3.

3. The weight and length of barley roots were not seriously affected by any of these added substances.

4. The weights of dry tops of barley were inversely proportional to the hydrogen-ion concentration of the solution, but the total range in the acidity of the cultures was comparatively small.

5. The growing barley plants exerted, in all cases, a neutralizing effect upon the reaction of the solution.

6. Over ninety percent of the phosphorus of the Knop's solution

was taken out of solution by the higher application of $Fe(OH)_3$, presumably by the formation of insoluble ferric phosphate. There was no evidence of poor phosphorus nutrition in this case; on the contrary, this was by far the best culture of barley, both in yield and in appearance.

7. Treatment with $Fe(OH)_3$ also produced neutrality of the nutrient medium, which may have been a factor contributing to the higher yield.

8. Peas, although deprived of a large portion of their cotyledons, had not yet developed significant differences in the various culture solutions during the growth period of twenty-one days.

9. With the proportions and kinds of solids and solutions obtaining here, there is no clear evidence of adsorption of nutrient ions.

This work was done in the Department of Agricultural Chemistry of the University of Wisconsin.

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UREDINALES OF GUATEMALA BASED ON COLLECTIONS BY E. W. D. HOLWAY

III. Puccinia, Exclusive of Species on Carduaceae

J. C. Arthur

The two previous instalments of the present account of the rusts of Guatemala were published in this Journal (June and October, 1918, pp. 325–336, 420–446), and one more part is to follow concluding with an index. The present part lists 76 species, of which twelve are described as new, six are placed under new combinations due to discovery of additional spore forms, and a number heretofore known from South America are now added to the North American flora.

Probably the most interesting group of rusts included in this part is that found on grasses. Grass rusts seem to be less common in the tropics than in the colder regions of the north, possibly because grasses are less abundant, at least where collectors go, or because these rusts are less conspicuous and so escape detection. The fine showing of fifteen species secured by Professor Holway, three being undescribed, is greatly to his credit as a close and discerning collector. Rather strangely, the two cosmopolitan rusts on corn and sorghum are not found in his material.

Even more satisfying than securing hitherto unknown species is the discovery made by Professor Holway of the probable connection of an aecial form on Eupatorium to go with one of the grass rusts on Aegopogon. It will be a slow process to connect the alternate forms of heteroecious species in the tropics, as the chances for making successive observations at the same locality and the opportunity to make cultures can come only at rare intervals. Professor Holway is to be congratulated on his fortunate find and clever observations in this direction.

Less interesting, but equally difficult for the taxonomist, are the Salvia rusts. Although autoecious, they are given to forming only uredinia, and run into endless modifications. Pycnia and aecia are especially rare. Not until numerous collections on every species of

host possible, taken at different periods and under diversified conditions of growth, are made available, can the taxonomic status of the various forms be reasonably well worked out. Dr. E. B. Mains has recently studied all the material in the writer's herbarium, and the seven species included in this article are in accordance with the results of this study.

The writer is deeply indebted to Professor Luigi Buscalioni, Director of the Royal Botanic Garden of Catania, Sicily, who is monographing the genus Saurauja, for his painstaking examination of the hosts for the two species of rusts on this genus. No fruit or flowers were present, making the task a difficult one. Thanks are also due to a number of American botanists who have given critical judgment upon the hosts of quite a number of collections. In general the hosts have been named for Professor Holway from phanerogamic specimens gathered at the same time as the rust specimens and submitted to various phanerogamic authorities.

102. PUCCINIA SORGHI Schw. (on Poaceae).

Euchlaena mexicana Schrad.

Zea Mays L.

A specimen of this rust, common wherever Indian corn is grown, was collected by Kellerman on *Zea Mays* at Guatemala City, Feb. 3, 1905, II, *5474*, and reported by Kern in Journ. Mycol. *l. c.* A specimen was also taken by him on *Euchlaena mexicana* at Guatemala City, Feb. 23, 1906, II, *5077*.

103. PUCCINIA PURPUREA Cooke (on Poaceae).

Sorghum vulgare Pers.

This common tropical rust was collected by Kellerman, at Antigua, Dept. Sacatépequez, Feb. 8, 1907, and issued in Kellerm. Fungi Sel. Guat. *16*.

104. PUCCINIA ANDROPOGONIS Schwein. (on Poaceae).

Andropogon condensatus H.B.K., San Lucas Toliman, 5100 feet alt., Dept. Solola, Feb. 2, 1915, II, III, *178*.

A common rust of the whole United States, having aecia on Castilleja and Pentstemon, but not before taken south of the border.

105. **Puccinia infuscans** Arthur & Holway sp. nov. (on Poaceae).

Imperata braziliensis Trin., Guatemala City, Jan. 3, 1915, *15*.

Uredinia hypophyllous, scattered, oblong or linear, 0.5–1 mm. long, soon naked, chestnut-brown, pulverulent, ruptured epidermis

evident; paraphyses none; urediniospores broadly ellipsoid or obovoid, 20–26 by 26–32 μ; wall dark cinnamon-brown, moderately thick, 2–3 μ, finely and closely verrucose, the pores 4, equatorial, distinct.

Telia hypophyllous, scattered, oblong or linear, 0.4–1 mm. long, early naked, chocolate-brown, ruptured epidermis evident; teliospores ellipsoid, 17–23 by 28–35 μ, wall chestnut-brown, 1.5–2 μ, lighter and thicker above, 5–7 μ, smooth; pedicel slightly tinted or colorless, once to twice length of spore, uniform diameter.

The host belongs to the tribe Andropogoneae. The urediniospores are similar to those of *Puccinia Ellisiana*, but very much larger. The species is readily distinguished from *P. rufipes* Diet. on *I. arundinacea*, which has paraphyses and echinulate urediniospores, and from *Uredo Imperatae* Magn. on *I. cylindrica* from Palestine, which has echinulate urediniospores, considerably thickened above.

106. PUCCINIA CHASEANA Arth. (on Poaceae).

Anthephora hermaphrodita (L.) Kuntze, Quirigua, Dept. Zacapa, March 22, 1916, II, III, *600*.

Heretofore this heteroecious rust has been known only from the West Indies. Aecia have not yet been detected.

107. PUCCINIA LEVIS (Sacc. & Bizz.) Magn. (on Poaceae).

Paspalum Humboldtianum Flügge, Solola, 7000 feet alt., Jan. 27, 1915, II, III, *129*; Guatemala City, Feb. 14, 1917, II, *864*.

A heteroecious rust for which no aecia have yet been found. It is found on a number of species of hosts from Texas and Louisiana through Mexico and the West Indies to Brazil and Argentina.

108. **Puccinia tubulosa** (Pat. & Gaill.) Arth. nov. comb. (on Poaceae).

Paspalum conjugatum Bergins, Quirigua, Dept. Zacapa, March 22, 1916, II, *594*.

Paspalum Humboldtianum Flügge, San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 11, 1915, II, *64*.

Paspalum paniculatum L., Quirigua, Dept. Zacapa, March 22, 1916, II, III, *595*.

Solanum torvum Swartz, Montufar, on the railway between Barrios and Guatemala City, Dec. 28, 1914, I, *0*.

A widespread tropical rust on many species of hosts. It is reported from Jamaica, Porto Rico, Cuba, and Bermuda of the West Indian Islands, and on the continent from Panama, Costa Rica, Mexico, and Texas.

Although often taken in the uredinial stage, when it has generally passed under the name of *Uredo paspalicola* P. Henn. (*U. Stevensiana* Arth.), yet the telia are not uncommon. Observations by Whetzel and Olive in Porto Rico and by Bethel in Panama, made it highly probable that the alternate form is *Aecidium tubulosum* on *Solanum torvum*. Cultures confirming this suggestion were made by Thomas in Porto Rico (Phytopathology **8**: 163, 1918).

The species was also collected by Kellerman on *Paspalum Humboldtianum*, El Rancho, Dept. Baja Vera Paz, Jan. 1, 1908, II, *8034*, and on *Axonopus compressus* (Swartz) Beauv., at Los Amates, Dept. Izabal, Feb. 22, 1908, II, *7540*.

109. **Puccinia macra** Arthur & Holway sp. nov. (on Poaceae).

Paspalum candidum (Humb. & Bonpl.) Kunth, Solola, 7000 feet alt., Jan. 31, 1915, II, III, *168*.

Uredinia chiefly hypophyllous, scattered or in small linear groups, round or elliptic, small, 0.5–1 mm. long, early naked, orange or yellowish, pulverulent; urediniospores ellipsoid, 23–29 by 28–35 μ; wall thin, 1–1.5 μ, pale yellow, finely and moderately or sparsely echinulate, the pores about 8, scattered.

Telia hypophyllous, scattered or sometimes crowded and confluent, elliptic or oblong, 0.5–1.5 mm. long, early naked, dark chestnut-brown, ruptured epidermis evident; teliospores ellipsoid or obovoid-ellipsoid, 23–28 by 35–48 μ, rounded at both ends or slightly narrowed below, slightly constricted at septum; wall chestnut-brown, 1.5–2 μ thick, slightly thicker at apex, 5–7 μ, smooth; pedicel tinted or nearly colorless, as long as the spore.

110. PUCCINIA ESLAVENSIS Diet. & Holw. (on Poaceae).

Valota insularis (L.) Chase (*Panicum insulare* Mey.), Laguna, Lake Amatitlan, Dept. Amatitlan, Feb. 8, 1915, II, *205*; Agua Caliente, Dept. Guatemala, Feb. 10, 1917, II, *857*.

A rust ranging from the southern border of the United States to Guatemala, the aecia for which are not known. It was collected by Kellerman on the same host in the same locality, Jan. 31, 1906, II, III, *5469*, and reported by Kern in Journ. Mycol. *l. c.*

111. PUCCINIA CYNODONTIS DeLac (on Poaceae).

Capriola dactylon (L.) Kuntze (*Cynodon dactylon* Pers.), Guatemala City, March 17, 1916, II, III, *592*; same, Feb. 14, 1917, II, *865*; Quirigua, Dept. Zacapa, March 22, 1916, II, *599*.

A common rust wherever the host grows, but the aecia, which occur on Plantago, have not been found in America.

112. PUCCINIA CENCHRI Diet. & Holw. (on Poaceae).

Cenchrus echinatus L., Guatemala City, March 17, 1916, II, iii, *591*.

Cenchrus viridis Spreng., Quirigua, Dept. Zacapa, March 22, 1917, II, *597*.

A common southern rust, for which the aecia are not known. It extends from the southern United States through Mexico and the West Indies.

113. PUCCINIA TRISETI Erikss. (on Poaceae).

Trisetum deyeuxioides (H.K.B.) Kunth, San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 7, 1915, II, III, *35*; same, Jan. 10, 1915, II, III, *58*.

A somewhat common rust from Colorado to Guatemala, for which the aecia are not known. It was collected by Kellerman on the same host, at Antigua, Feb. 13, 1905, II, III, *5322*.

114. PUCCINIA DOCHMIA Berk. & Curt. (on Poaceae).

Muhlenbergia ciliata (H.B.K.) Kunth, San Rafael, 6800 feet alt., Dept. Guatemala, Jan. 9, 1915, III, *53*.

The species ranges from central Mexico to Costa Rica. Its aecia are not yet known. It was collected by Kellerman near Antigua, on *M. quitensis* (H.B.K.) Hitchc., Feb. 3, 1908, *7196*, *7190*.

115. PUCCINIA JAMESIANA (Peck) Arth. (on Poaceae).

Bouteloua filiformis (Fourn.) Griffiths, Chile, on railway between Guatemala City and Barrios, Feb. 12, 1915, III, *206*.

A heteroecious rust with aecia on various asclepiadaceous genera, common in the United States, but much less so southward.

116. PUCCINIA EPIPHYLLA (L.) Wettst. (on Poaceae).

Poa annua L., San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 8, 1915, II, III, *36*.

It is noteworthy that this heteroecious rust, often listed as *P. Poarum* Niels., here shows telia as well as uredinia, although nowhere east of the Rocky Mountains in the United States, where it is common, are telia known. Furthermore, on this host no telia have previously been taken, although known from various collections ranging through Mexico, California, and northward to Oregon. Other hosts in the mountains show telia. The aecial hosts are Tussilago and Petasites, all the aecial species being natives of northern regions.

117. **Puccinia Aegopogonis** Arthur & Holway sp. nov. (on Poaceae and Carduaceae).

Eupatorium Mairetianum DC., Solola, Jan. 25, 1915, O, I, *113*.

Eupatorium sp., Guatemala City, Dec. 23, 1916, O, I, *631*; same, Feb. 14, 1917, O, I, *868*; Huehuetenango, Jan. 21, 1917, O, I, *754*.

Aegopogon cenchroides Humb. & Bonpl., San Rafael, 7000 feet alt., Dept. Guatemala, Jan 9, 1915, II, III, *54* (type); Solola, 7000 feet alt., Jan. 31, 1915, ii, III, *164*; Antigua, Dept. Sacatépequez, Dec. 28, 1916, II, III, *650*; Huehuetenango, Jan. 21, 1917, III, *760*; Guatemala City, Feb. 14, 1917, II, *869*.

Aegopogon tenellus (Cav.) Trin., San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 8, 1915, ii, III, *37*.

Uredinia hypophyllous, scattered, oblong-linear, 0.2–0.5 mm. long, cinnamon-brown, pulverulent, early naked, ruptured epidermis noticeable; urediniospores globoid or broadly ellipsoid, 21–25 by 23–29 μ; wall golden- or light cinnamon-brown, thin, 1–1.5 μ, finely and closely echinulate, the pores 6–8, scattered.

Telia hypophyllous, like the uredinia in size and distribution, chocolate-brown, early naked; teliospores broadly ellipsoid, 19–24 by 25–30 μ, rounded above and below, slightly or not constricted at the septum, which is often oblique; wall chestnut-brown, moderately thin, about 1.5 μ, darker and slightly thicker at apex, 3–5 μ, smooth; pedicel yellowish or colorless, once to twice length of spore.

The species differs from *Uromyces Aegopogonis* Diet. & Holw. (*Nigredo Aegopogonis* Arth.) by having in general somewhat larger urediniospores, and in having two-celled instead of one-celled teliospores. The two forms, or so-called species, are undoubtedly to be considered races of one and the same species.

The same kind of aecia have been found associated with both forms in such intimate and unmistakable relation that no hesitation is longer felt in connecting them with this species, although no opportunity has yet presented itself to confirm the observations by cultures. The aeciospores have the somewhat uncommon character of thickened wall above, which readily separates them from the aecia of *Puccinia Eleocharidis* Arth. on the same genus of hosts, which have a uniformly thickened wall.

The aecia were described as *Aecidium roseum* Diet. & Holw., from a collection made at Eslava, near the City of Mexico, Oct. 3 or 4, 1896. The type collection of *Uromyces Aegopogonis* Diet. & Holw.

was also taken near the City of Mexico, Oct. 1, 1896. A collection of aecia was made at Amecameca, near the City of Mexico, on Oct. 20, 1903, and of telia Oct. 22, 1903. Collections of both aecia and telia were made at Patzcuaro, Oct. 19, 1898. Many other collections were made of both stages in these and nearby localities as well as elsewhere in central Mexico.

It was not, however, until Prof. Holway was collecting in Guatemala in January, 1917, that the genetic connection of the two stages was clearly suspected. On Jan. 21, of this year, at Heuhuetenango, a clump of some shrubby Eupatorium was found completely covered with *Aecidium roseum* (no. 754). It was at the bottom of a high bank, with *Aegopogon cenchroides* on the bank above, well rusted with *Puccinia Aegopogonis* (no. 760), and no other sedge or grass rust in the vicinity. The same close and well isolated association was again found on Feb. 14, 1917, near Guatemala City (nos. 868 and 869).

The circumstantial evidence is both direct and abundant, indicating that the apically thickened aeciospores on Eupatorium go genetically to the Aegopogon rust, and that they are not part of the rust on Eupatorium, which since 1906 has often been called *Puccinia rosea*, but should go under the name *P. Conoclinii* Seym. The name *Aecidium roseum* Diet. & Holw., should technically be entered as a synonym under *Uromyces Aegopogonis*.

The rust was collected by Kellerman, on phanerogamic specimens now in the National Herbarium, of *A. cenchroides*, Santa Maria, Dept. Quezaltenango, Feb. 5, 1905, II, *5572*, and Cerro Quemada, Dept. Quezaltenango, Feb. 8, 1906, III, *5932*, *5948*.

118. **Puccinia subdigitata** Arthur & Holway sp. nov. (on Poaceae).

Brachypodium mexicanum (Roem. & Schult.) Link, San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 7, 1915, II, III, *23*.

Uredinia amphigenous, scattered, elliptic, small, about 0.5 mm. long, early naked, yellowish, pulverulent, ruptured epidermis inconspicuous; paraphyses few, oblong or clavate, 10–15 by 26–35 μ, the wall pale cinnamon-brown, 1–2 μ thick; urediniospores globoid or broadly ellipsoid, 12–15 by 14–19 μ; wall thin, 1 μ or less, pale yellow or colorless, finely and closely echinulate, the pores obscure, probably scattered.

Telia chiefly hypophyllous, crowded but seldom confluent, elliptic or oblong, 0.5–1 mm. in length, dark gray, long covered by the epidermis bordered by a thin layer of dark brown stromal hyphae; teliospores oblong or clavate-oblong, 10–16 by 27–45 μ, the apex truncate,

smooth or with a few, 1–5, short digitate projections, usually somewhat narrowed below, slightly or not constricted at septum; wall dark chestnut-brown above, paler below, thin, about 1 μ, slightly thickened at apex, 3–7 μ including projections; pedicel very short, tinted.

On Brachypodium there also occur *Puccinia Baryi*, which has teliospores with smooth apices, and *P. himalensis*, which has coronate teliospores, but the coronations are more prominent and the uredinio-spores much larger than in the present form.

119. PUCCINIA CYPERI Arth. (on Cyperaceae).

Cyperus hermaphroditus (Jacq.) Standley, San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 8, 1915, II, III, *136 A*.

Cyperus sp., Quirigua, Dept. Zacapa, March 22, 1916, II, III, *598*.

Kyllinga odorata Vahl, Panajachel, Dept. Solola, Jan. 3, 1917, II, *663*.

A common heteroecious rust of North America. It is here considered distinct from the somewhat more common *P. canaliculi*, having aecia on Ambrosia and Xanthium.

120. PUCCINIA ELEOCHARIDIS Arth. (on Cyperaceae).

Eleocharis geniculata (L.) R. Br.

Eleocharis sp.

A collection was made by Kellerman on *E. geniculata*, at Palmar, Dept. Quezaltenango, Feb. 11, 1916, II, *5419*, and reported by Kern in Mycologia *l. c.*; and on an undetermined Eleocharis, at Laguna, Lake Amatitlan, Feb. 5, 1905, II, *5365*, and also at the same place, Feb. 17, 1906, II, *5399*.

The species is abundant in north temperate America, where the aecia occur on species of Eupatorium, but in tropical America only uredinia are known.

121. PUCCINIA CARICIS-POLYSTACHYAE Diet. (on Cyperaceae).

Carex polystachya Wahl. (*C. cladostachya* Wahl.), San Rafael, Dept. Guatemala, Jan. 10, 1915, ii, III, *55*; Solola, Dept. Guatemala, Jan. 27, 1915, ii, III, *124*; Guatemala City, Dec. 20, 1916, II, III, *611*.

A long-cycle, heteroecious rust, whose aecia and alternate host are unknown. The species was originally collected in southern Mexico by Professor Holway (no. 3727). The Guatemalan material here

listed was recently described as a new species by Kern, *P. Kellermanii* (Mycologia 9: 210. 1917), but a careful comparison seems to indicate that only one species is involved. The pores of the urediniospores are often indistinct, and appear to vary from 2 to 4, always equatorial.

122. PUCCINIA PALLOR Arth. & Holw. (on Amaryllidaceae).

Bomaria acutifolia Herb., Volcan de Agua, Dept. Sacatépequez, Jan. 13, 1915, II, III, *84*; same, March 7, 1916, O, I, II, III, *562*.

An Eriosporangium-like species with all spore forms. It occurs also in Costa Rica.

123. PUCCINIA CANNAE (Wint.) P. Henn. (on Cannaceae).

Canna sp., Mazatenango, Dept. Suchitepequez, Feb. 22, 1916, II, *516*.

A common long-cycle rust of the tropics, whose pycnia have not been seen. Whether pycnia are associated with aecia or with primary uredinia can not be predicted. It was collected by Kellerman at Mazatenango, Feb. 28, 1905 (Kellerm. Fungi Sel. Guat. *3*).

124. PUCCINIA POLYGONI-AMPHIBII Pers. (on Polygonaceae).

Persicaria (*Polygonum*) sp.

A collection was made by Kellerman at Laguna, Lake Amatitlan, Jan. 25, 1906, II, *5392*, and reported by Kern in Mycologia *l. c.* This long-cycle species is not uncommon in the tropics, where only uredinia are generally found.

125. PUCCINIA PUNCTIFORMIS Diet. & Holw. (on Polygonaceae).

Rumex crispus L., Solola, 6500 feet alt., Jan. 25, 1915, II, *116*; Panajachel, Dept. Solola, Jan. 3, 1917, II, III, *676*.

A long-cycle species, whose aecia are not known. It ranges from Berkeley, Calif., southward through Mexico, into Central America.

126. PUCCINIA DETONSA Arth. & Holw. (on Caryophyllaceae).

Stellaria ovata Willd., Colomba, Dept. Quezaltenango, Feb. 3, 1917, *824*.

A short-cycle species, occurring on the same host in Costa Rica. The spores have thinner walls than in *P. Arenariae*.

127. PUCCINIA MODICA Holw. (on Caryophyllaceae).

Arenaria alsinioides Willd., Volcan de Agua, Dept. Sacatépequez, March 4, 1916, II, iii, *561*.

Arenaria lanuginosa Rohrb., Solola, 7000 feet alt., Jan. 25, 1915, II, III, *111*.

This long-cycle rust has heretofore been known only from Mexico. Most of the teliospores in the collection number 561 are mesosporic. Re-examination of the type collection shows the presence of a few mesospores, but they are not mentioned in the published description. The other collection here listed also has only a few mesospores. Apparently such one-celled teliospores are most abundantly produced in sori first arising when telial production begins. The first stage of this rust is yet unknown.

128. PUCCINIA FOVEOLATA (Berk. & Curt.) P. Henn. (on Anonaceae).
Xylopia sp.

This peculiar short-cycle rust, first found in Surinam and named *Dasyspora foveolata*, and later found in other parts of South America, was collected by Kellerman, at Los Amates, Dept. Izabal, March 15, 1905, *5330*, and reported by Kern in Mycologia *l. c.* under the often used name *Puccinia gregaria* Kunze.

129. **Puccinia circinata** (Schwein.) comb. nov. (on Malpighiaceae).
Genus and species undetermined.

Through the kindness of the Director of the Royal Botanic Gardens, Kew, the writer has recently been able to examine a part of the type collection of *Uredo circinata* Schwein., published by Berkeley and Curtis in their account of "Exotic fungi from the Schweinitzian herbarium, principally from Surinam," Journ. Acad. Phila. **2**: 282. 1853. It was stated to be "on the leaves of some unknown plant."

A collection made by Kellerman, at Gualan, Dept. Zacapa, Guatemala, Dec. 28, 1905, *5457*, on the leaves of some plant, whose identity was not ascertained, shows uredinia and telia, and the uredinia exactly correspond to those of the rust from Surinam. The urediniospores are very distinctive on account of the conspicuously long echinulations. In the comments attached to the original description it is noted that "the globose spinulose spores are the distinctive mark of this fine species; the processes are nearly as strong as in [the teliospores of] *Puccinia aculeata* Schweinitz." It required, however, another spore form to make evident the systematic position of the rust, and to rescue the name from the limbo of *species dubiae*.

The species has the same general characteristic as *Puccinia inflata* Arth., on species of Stigmaphyllon in the West Indies. In both species the bulbous inflation of the pedicel next to the spore is a curious and marked feature. From *P. inflata*, however, it is abundantly distinct,

but we may fairly infer that the hosts of both the Guatemalan and Surinam collections are species of Stigmaphyllon, or at least that they belong to the Malpighiaceae. The rust may be characterized as follows:

Uredinia amphigenous, scattered or in groups 2–3 mm. across, round or oval, 0.3–1 mm. across, rather tardily naked, pulverulent, cinnamon-brown, ruptured epidermis conspicuous; urediniospores when dry or in alcohol broadly ellipsoid or globoid, 22–26 by 26–32 μ; wall golden or dark cinnamon-brown, 2.5–3 μ thick, very sparsely and prominently echinulate, the echinulations 2.5 μ long, 1 μ wide at base, 7–10 μ apart, and colorless, the pores 2–4, equatorial; urediniospores when in water swelling to 27–32 by 32–40 μ; wall 5–7 μ thick, with a cinnamon-brown inner layer 3–4 μ thick and a colorless outer layer 1.5–4 μ thick.

Telia amphigenous, scattered, blackish-brown; teliospores broadly ellipsoid, 26–35 by 35–50 μ, rounded at both ends, slightly or not constricted at septum; wall dark chestnut-brown, uniformly 3–4 μ thick, rather obscurely reticulate, the areolae about 2–3 μ across; pedicel usually attached obliquely, once to once and a half length of spore, in water forming a globoid swelling next the spore up to 26 μ in diameter.

130. PUCCINIA EUPHORBIAE P. Henn. (on Euphorbiaceae).

Aklema caracasana (Klotzsch & Garcke) Millsp. (*Euphorbia caracasana* Boiss.), Guatemala City, Dec. 31, 1914, II, III, *3*; Antigua, Dept. Sacatépequez, Jan 11, 1915, ii, III, *66*; same, Dec. 27, 1916, II, III, *641*; Quezaltenango, Jan. 18, 1917, III, *740*.

A long-cycle rust, having pycnia, uredinia, and telia, with large and highly characteristic teliospores. It is the form designated by the Sydows as variety *longipes*, but agrees with the type material from Abyssinia. It was also collected by Kellerman, on *Aklema cotinifolia* (L.) Millsp. (*Euphorbia cotinifolia* L.), at San Lucas, Dept. Solola, Feb. 16, 1906, II, III, *5433*. It occurs in Mexico and the West Indies.

131. **Puccinia velata** (Ellis & Everh.) comb. nov. (on Euphorbiaceae).

Aklema Scotana (Schlecht.) Millsp. (*Euphorbia Scotana* Boiss.)

A phanerogamic specimen of this host in the Field Museum at Chicago, Ill., sheet number 195471, shows the rust named. It was collected at San Lucas, Dept. Solola, Feb. 16, 1906, by Kellerman, *5433*, and there are both uredinia and telia present.

The species is listed in Sydow's Monog. Ured. 1: 457, as *P. Euphorbiae minor* Diet. & Holw., described in 1897, and credited to Mexico, but it is also identical with *Uredo velata* Ellis & Everh. (Bull. Torrey Bot. Club 22: 435. 1895), on *Chamaesyce cordata* (Meyer) Millsp. (*Euphorbia cordata* Meyer), from Hawaii. The type specimen of *Uredo velata* has been examined and shows telia as well as uredinia. The species is closely related to the preceding one, but is readily distinguished by the broader and shorter teliospores, and the shorter pedicels.

132. PUCCINIA ARECHAVELATAE Speg. (on Sapindaceae).

Cardiospermum coluteoides H.B.K., Progreso, between Guatemala City and Barrios, Feb. 12, 1915, *208*.

A short-cycle rust, very common in the tropics. The host of this collection has not before been reported. The species was collected by Kellerman, on *C. grandifolium* Swartz, at El Rancho, Dept. Jalapa, Jan. 6, 1906, *5401*, and reported by Kern in Journ. Mycol. *l. c.*

133. PUCCINIA GOUANIAE Holw. (on Frangulaceae).

Gouania lupuloides (L.) Urban (*G. domingensis* L.), Escuintla, Feb. 17, 1916, II, *500*.

A long-cycle species, having pycnia, uredinia, and telia, known from Cuba, Porto Rico, and Panama.

134. PUCCINIA INVAGINATA Arth. (on Frangulaceae).

Gouania lupuloides (L.) Urban (*G. domingensis* L.), Escuintla, Feb. 17, 1916, ii, III, *497*.

Gouania sp., Mazatenango, Feb. 21, 1916, II, *518*; Guatemala City, Dec. 21, 1916, ii, III, *614*; Moran, Dept. Amatitlan, Dec. 22, 1916, ii, III, *618*.

The rust is a long-cycle form, for which the primary stage is unknown, but it doubtless has pycnia and no aecia. It was first collected on the island of St. Croix in 1896, and named *Uredo Gouaniae* Ellis & Kelsey. Since then it has been taken a number of times in Porto Rico, always in the uredinial stage. The telia have heretofore been known only from Cuba. The teliospores are very similar to those of *P. Gouaniae* Holw., only slightly larger, but the one-pored, peculiarly shaped urediniospores are quite unlike those of that species.

135. PUCCINIA HETEROSPORA Berk. & Curt. (on Malvaceae).

Abutilon discissum Schlecht., Zunil, Dept. Quezaltenango, Jan. 28, 1917, *782*.

Abutilon sp., Aguas Amargas, Dept. Quezaltenango, Jan. 30, 1917, *797, 798.*

Malvaviscus arboreus Cav., Mazatenango, Feb. 25, 1916, *529;* San Felipe, Dept. Retalhuleu, Jan. 12, 1917, *694.*

Malvaviscus sp., Chinautla, Dept. Guatemala, Feb. 12, 1916, *485.*

Sida cordifolia L., Sanarate, Dept. Guatemala, Feb. 10, 1916, *474.*

A very common short-cycle rust of tropical regions, notable for its abundant production of mesospores, and its numerous hosts. It was also collected by Kellerman on *Sida cordifolia*, at Gualan, Dept. Zacapa, Jan. 23, 1905, *4323*, and again at the same place, March 12, 1905, *4323bis* (Kellerm. Fungi Sel. Guat. *6*).

136. PUCCINIA SHERARDIANA Körn. (on Malvaceae).

Sida spinosa L., Chinautla, Dept. Guatemala, Feb. 12, 1916, *488.*

A short-cycle species with large golden-brown sori and terete teliospores, somewhat resembling *P. Malvacearum*. It occurs abundantly in western North America, and ranges southward along the mountains to Peru.

137. PUCCINIA EXILIS Syd. (on Malvaceae).

Pavonia rosea Schlecht., Quirigua, Dept. Zacapa, March 22, 1916, *593.*

A short-cycle rust, resembling *P. heterospora* but with much smaller teliospores and seemingly without mesospores. This is the first record for North America. It has heretofore been known only from Brazil.

138. PUCCINIA FILOPES Arth. & Holw. (on Sterculiaceae).

Buettneria lateralis Presl, Escuintla, Feb. 17, 1916, *501.*

A short-cycle species for which this number is the type, occurring also in Costa Rica.

139. **Puccinia vergrandis** Arthur & Holway sp. nov. (on Dilleniaceae).

Saurauja pauciserrata Hemsl., Colomba, Dept. Quezaltenango, Feb. 2, 1917, ii, III, *820.*

Uredinia not seen; urediniospores in the telia, obovoid, 26–32 by 32–40 μ; wall golden-brown, thick, 3 μ, somewhat thicker above up to 5 μ, coarsely and moderately echinulate, the pores 2–4, approximately equatorial or slightly superequatorial.

Telia chiefly hypophyllous, confluent in annular groups 2–4 mm. across, round, 0.3–1 mm. in diameter, early naked, chestnut-brown becoming cinereous from germination, ruptured epidermis evident;

teliospores ellipsoid or oblong, 29–31 by 37–45 μ, rounded above, blunt below, slightly constricted at septum; wall golden- or cinnamon-brown, rather thick, 1.5–2.5 μ, thicker above, 4–7 μ, rugosely verrucose; pedicel colorless, fragile.

The species appears like a short-cycle form, and the presence of urediniospores does not necessarily exclude that possibility. Sectioning for pycnia failed to disclose the presence of such a stage.

140. **Puccinia aucta** Arthur & Holway sp. nov. (on Dilleniaceae).

Saurauja Conzatti Busc. (?), Chinautla, Dept. Guatemala, Jan. 17, 1915, *90*; Guatemala City, Dec. 20, 1916, *608*.

Saurauja Smithiana Busc. (?), Huehuetenango, Jan. 23, 1917, *775*; Colomba, Dept. Quezaltenango, Feb. 2, 1917, *819*; road between Colomba and Quezaltenango, Feb. 4, 1917, *830* (type).

Saurauja sp., Guatemala City, Feb. 8, 1917, *844*.

Telia hypophyllous, crowded in small mostly confluent groups 0.5–2 mm. across on somewhat larger purple spots, round, 0.1–0.5 mm. in diameter, early naked, pulvinate, chocolate-brown, ruptured epidermis inconspicuous; teliospores oblong, 10–15 by 32 42 μ, rounded or obtuse at both ends, often narrowed below, somewhat constricted at septum; wall light chestnut-brown, or lighter after germination, 1.5–2 μ thick, much thicker above, 3–10 μ, smooth; pedicel light brown to colorless, once to twice length of spore or shorter, persistent.

A short-cycle rust, causing abundant spotting on both sides of the leaves. Probably no pycnia are formed. Germination takes place readily *in situ* at maturity. The several collections appear to belong possibly to two or more species of host, as the leaves vary from smooth to strongly pubescent.

141. PUCCINIA VIOLAE (Schum.) DC. (on Violaceae).

Viola nannei Polak., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, III, *566*; same, Dec. 29, 1916, II, III, *656*.

A long-cycle rust, common on violets in both America and Europe, having somewhat variable characters.

142. PUCCINIA CUPHEAE Holw. (on Lythraceae).

Cuphea Hookeriana Walp., Solola, 5500 feet alt., Jan. 27, 1915, *133*.

A short-cycle species, rather common in Mexico and Central America.

143. PUCCINIA FUCHSIAE Syd. & Holw. (on Onagraceae).

Fuchsia microphylla H.B.K., Quezaltenango, Jan. 19, 1917, *753*.

Lopezia hirsuta Jacq., San Rafael, Dept. Guatemala, Jan. 9, 1915, *34*; Antigua, Dept. Sacatépequez, Dec. 28, 1916, *647* (with some *Uredo Fuchsiae*).

A short-cycle rust, heretofore known only from the type collection by Professor Holway, made in central Mexico.

144. PUCCINIA HYDROCOTYLES (Link) Cooke (on Ammiaceae).

Hydrocotyle Bonariensis Lam., San Lucas Toliman, 5100 feet alt., Dept. Solola, Feb. 3, 1915, II, *190*.

Hydrocotyle mexicana Schlecht. & Cham., San Lucas Toliman, 6500 feet alt., Dept. Solola, Feb. 3, 1915, II, *189*.

A common long-cycle rust of both North and South America. Pycnia have not yet been found, and aecia probably do not occur in the life cycle. The aecia on Hydrocotyle that have been referred here are doubtless heteroecious.

145. **Puccinia Arracacharum** (Lindr.) Arth. comb. nov. (on Ammiaceae).

Arracacia bracteata Coult. & Rose, Volcan de Agua, Dept. Sacatépequez, Jan. 13, 1915, O, I, II, III, *86*; same, March 7, 1916, O, I, II, iii, *558*.

This distinctive species is Eriosporangium-like in its general combination of characters, and especially in having aecia without peridia, and pale teliospores germinating at maturity in the sorus. It is quite unlike *P. imperspicua* Syd. on another species of Arracacia from Mexico, a species without uredinia, with a peridium in the aecium, and with thicker-walled teliospores.

The species was described by Lindroth in 1891 (Medd. Stockholms Högsk. Bot. Inst. **4**: 1, 5) from collections made by Lagerheim in Ecuador, S. A. During two years' observation Lagerheim did not find the aecia, which were very common, to be followed by uredinia and telia (*cf.* Lindroth, Die Umbelliferen-Uredineen, Act. Soc. Faun. Fl. Fenn. **22**[1]: 142. 1902), and their genetic association was considered doubtful. The aecia were therefore described as *Caeoma Arracacharum* (*l. c.*, p. 1), and the uredinia and telia as *Puccinia Arracachae* (*l. c.*, p. 5), in which disposition the Sydows concurred (Monogr. Ured. **1**: 360. 1902). In the Guatemalan collections by Professor Holway, cited above, all spore forms occur on the same leaves. More-

over, there is a remarkable and significant similarity in the size and sculpturing of the aeciospores and urediniospores, not taken into account in the published comments on the South American material, although to be seen in the type material as well as in that from Guatemala. For convenience a full technical description is appended. The generic name of the host was written Arracacha at one time, but is now accepted in the form Arracacia.

Pycnia chiefly epiphyllous, few, subepidermal, chestnut-brown, globoid, 150–220 μ in diameter.

Aecia amphigenous, in groups of 2–6 with the pycnia, 0.1 μ or less in diameter; peridium wanting, the sorus bounded by a thin layer of mycelium; aeciospores angularly ellipsoid or oblong, 18–23 by 24–35 μ; wall colorless, 2–2.5 μ thick, variably verrucose, sometimes appearing echinulate.

Uredinia hypophyllous, scattered, round, 0.2–0.5 mm. across, early naked, pulverulent, yellow or pale cinnamon-brown, ruptured epidermis evident; urediniospores ellipsoid or obovoid, 18–24 by 29–37 μ; wall colorless or yellowish, moderately thick, 1.5–2.5 μ, closely echinulate, the pores obscure.

Telia hypophyllous, scattered, round, 0.4–0.6 mm. across, early naked, pulvinate, chestnut-brown, ruptured epidermis inconspicuous, germinating readily in the sorus; teliospores ellipsoid or oblong, 21–29 by 34–51 μ, round at both ends, or slightly narrowed below, little constricted at septum; wall cinnamon-brown, thin, 1–2 μ, much thicker above, 5–10 μ, smooth; pedicel colorless, once and a half length of spore or less, tapering downward.

146. **Puccinia obscurata** Arthur & Holway sp. nov. (on Ammiaceae).

Neonelsonia ovata Coult. & Rose, Volcan de Agua, Dept. Sacatépequez, March 4, 1916, II, III, *555*.

Uredinia chiefly hypophyllous, scattered, round or oval, 0.1–0.8 mm. across, early naked, pulverulent, pale cinnamon-brown, ruptured epidermis evident; urediniospores globoid or obovoid, 18–26 by 24–29 μ; wall colorless or light-yellow, moderately thick, 1.5–2.5 μ, closely echinulate, the pores rather indistinct, 2–3, usually 2, equatorial.

Telia hypophyllous, scattered, round, 0.1–0.3 mm. across, early naked, somewhat pulverulent, chestnut-brown, ruptured epidermis evident; teliospores broadly ellipsoid or oblong, 23–31 by 30–40 μ, rounded at both ends, slightly or not constricted at septum; wall cinnamon-brown, thin, 1–1.5 μ, thicker above, 3–7 μ, smooth; pedicel colorless, longer than the spore, fragile, and usually broken away.

The microscopic appearance of this rust gives much the same impression as that of *P. Arracacharum*, on Arracacia, but the detailed

characters are quite dissimilar. Coulter and Rose (Contr. U. S. Nat. Herb. 3: 306) consider the host of this rust to be somewhat like Arracacia and Smyrnium, but not closely allied to any genus of the family. The life cycle could not be completed from the material in hand. The germination of the teliospores does not seem to take place in the sorus at maturity.

147. PUCCINIA OBLIQUA Berk. & Curt. (on Asclepiadaceae).

Philibertella crassifolia Hemsl., Laguna, Lake Amatitlan, Feb. 8, 1915, *197*, *198*.

Asclepiad vine, San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 9, 1915, *45*; same, between San Lucas Toliman and Patalul, Feb. 4, 1915, *192*; Mazatenango, Feb. 21, 1915, *519*.

An exceedingly variable short-cycle species in both gross and microscopic characters. The two collections from Lake Amatitlan are quite unlike in gross appearance. Number 198 has large, compact sori, each often with a ring of smaller sori about it, in contrast to the groups of numerous, small, pulvinate sori generally seen. This form with large sori corresponds to a similar form of *Puccinia Asteris*, which was once given the name of *P. monoecia*. The same species was collected by Kellerman on *P. crassifolia* at Laguna, Lake Amatitlan, Feb. 11, 1905, *4348* (Kellerm. Fungi Sel. Guat. *5*), and again at the same place Jan. 20, 1906, *5437*, and reported by Kern in Journ. Mycol. *l. c.* under the name of *P. Cynanchi* Lagerh., a name now treated as a synonym.

148. PUCCINIA MARSDENIAE Diet. & Holw. (on Asclepiadaceae).

Marsdenia mexicana Decaisne, San Lucas Toliman, 5100 feet alt., Dept. Solola, Feb. 2, 1915, II, iii, *171*; same, Jan. 4, 1917, II, III, *677*.

This striking rust was previously known only from the type locality at Cuernavaca, Mexico. Its initial stage has not yet been seen.

149. PUCCINIA NOCTICOLOR Holw. (on Convolvulaceae).

Ipomoea fistulosa Mart., Mixco, Dept. Guatemala, Jan. 9, 1915, I, III, *40*.

This long-cycle rust has heretofore been known only from a number of collections on *I. intrapilosa*, all made by Professor Holway in Mexico. No pycnia are yet known, and uredinia do not occur. The aeciospores are sometimes thickened up to 12 μ above. The species has been published as *Allodus nocticolor* (Holw.) Orton.

150. PUCCINIA CRASSIPES Berk. & Curt. (on Convolvulaceae).

Ipomoea glabriuscula House, Sanarate, Dept. Guatemala, Feb. 10, 1916, i, III, *472*; Agua Caliente, Dept. Guatemala, Feb. 10, 1917, I, III, *856*.

Ipomoea tiliacea (Willd.) Choisy (*I. fastigiata* Sweet), Guatemala City, Jan. 3, 1915, I, *11*; Laguna, Lake Amatitlan, Feb. 8, 1915, I, III, *201*.

151. PUCCINIA DICHONDRAE Mont. (on Dichondraceae).

Dichondra sericea Swartz, San Rafael, Dept. Guatemala, Jan. 9, 1915, *50*.

A short-cycle rust occurring from the southern United States, southward into South America, and also in Australia.

152. PUCCINIA FUMOSA Holw. (on Polemoniaceae).

Loeselia ciliata L., Palin, Dept. Amatitlan, Dec. 24, 1916, II, *636*.

Loeselia glandulosa G. Don., San Lucas Toliman, 6500 feet alt., Dept. Solola, Feb. 3, 1915, ii, III, *184*.

A long-cycle rust with all spore stages, heretofore reported only from Mexico.

153. PUCCINIA HELIOTROPII Kern & Kellerm. (on Heliotropiaceae).

Heliotropium indicum L., Sanarate, Dept. Guatemala, Feb. 10, 1916, *468*.

A short-cycle rust, heretofore known only from Kellerman's two collections on the same host from Gualan, Dept. Zacapa, March 12, 1905, *4326*, and Dec. 30, 1905, *5422*, reported by Kern in Journ. Myc. *l. c.* where the species is described, and also issued in Kellerm. Fungi Sel. Guat. *15*.

154. **Puccinia gilva** Arthur & Holway sp. nov. (on Heliotropiaceae).

Heliotropium physocalycinum Donn. Smith, Moran, Dept. Amatitlan, Dec. 22, 1916, II, III, *626*; Antigua, Dept. Sacatépequez, Dec. 30, 1916, II, III, *658* (type).

Uredinia hypophyllous, scattered, round, 0.2–0.6 mm. across, early naked, pulverulent, dirty white, ruptured epidermis noticeable; urediniospores obovoid, 16–19 by 19–23 μ; wall light yellow or colorless, thin, 1–2 μ, closely echinulate, with the pores obscure.

Telia hypophyllous, aggregated or scattered, often crowded and circinating about a uredinium, round, pulvinate, early naked, 0.1–0.3 mm. across, pale brown, often cinereous from germination, ruptured epidermis inconspicuous; teliospores oblong, 16–22 by 40–47 μ, obtuse above, obtuse or somewhat narrowed below, slightly constricted at

septum; wall yellowish to pale golden-brown, 1–1.5 μ thick, much thicker at apex, 4–7 μ, smooth; pedicel colorless, short, fragile.

A species having the appearance of a leptoform, but with uredinia unmistakably associated with the telia. No trace of pycnia or aecia could be found. It has paler and thinner-walled spores than the short-cycled *P. Heliotropii* K. & K., and larger teliospores than *P. heliotropicola* Speg.

155. PUCCINIA CORDIAE (P. Henn.) Arth. (on Ehretiaceae).

Cordia gerascanthus L. (*C. alliodora* Cham.), Escuintla, Feb. 18, 1916, O, II_1, ii_2, III, *503*; same, Feb. 19, 1916, ii_2, III, *508*.

At the time this species was described by the writer (Mycologia **8**: 17. 1916), no collection answering to Hennings' description of *Uredo Cordiae* (Hedwigia **43**: 163. 1904) had been seen. Number 503 of Professor Holway's material bears all spore forms from pycnia to telia belonging to the species, thus completing our knowledge of the life cycle. The primary uredinia correspond exactly to Hennings' description, which brings his name into synonymy. Primary uredinia are also shown in Sydow's Uredineen *2098*. They differ from secondary uredinia in having slightly larger urediniospores, in absence of paraphyses, and in causing marked hypertrophy, as well as in the presence of pycnia. The pycnia are amphigenous or caulicolous, in groups or scattered over more or less deformed terminal leaves, conspicuous, and subepidermal. The secondary uredinia are scattered over the under side of the leaf, without causing hypertrophy. They have hyphoid, peripheral paraphyses, and, of course, are not accompanied by pycnia.

156. PUCCINIA URBANIANA P. Henn. (on Verbenaceae).

Cornutia grandifolia Schauer, Colomba, Dept. Quezaltenango, Feb. 2, 1917, *822*.

A new host for this short-cycle and very common tropical rust.

157. PUCCINIA ELATIPES Arth. & Holw. (on Verbenaceae).

Lippia myriocephala Schlecht. & Cham., San Lucas Toliman, Dept. Solola, Jan. 4, 1917, O, ii, III, *678*; road between Quezaltenango and Colomba, Feb. 4, 1917, O, ii, III, *831*.

A long-cycle species having pycnia, uredinia, and telia, also found in Costa Rica, for which the Guatemalan collection *831* is the type.

158. PUCCINIA FARINACEA Long (on Lamiaceae).

Salvia amarissima Orteg., Antigua, Dept. Sacatépequez, March 1–2, 1916, O, I, II, III, *547*.

Salvia elegans Vahl, Solola, Jan. 28, 1915, III (with *P. delicatula*), *140a*; Sija, Dept. Quezaltenango, Jan. 26, 1917, ii, III, *780*.

Salvia Holwayi Standley, Quezaltenango, Jan. 18, 1917, II, III, *741*.

Salvia lavanduloides H.B.K., Solola, Jan. 30, 1915, II, *165*; Antigua, Dept. Sacatépequez, Dec. 28, 1916, II, *654*.

Salvia Lindenii Benth., Volcan de Agua, Dept. Sacatépequez, Jan. 13, 1915, II, iii, *88*; same, March 7, 1916, II, III, *580*; road between Quezaltenango and Colomba, Feb. 4, 1917, II, *833*.

Salvia nepetoides H.B.K., Quezaltenango, Jan. 20, 1915, ii, III, *94*.

Salvia sp., Huehuetenango, Jan. 23, 1917, O, I, ii, III, *773*; same, Jan. 24, 1917, O, I, II, III, *777*.

The most common Salvia rust of Mexico and Central America, extending northward into the United States.

Until now the life cycle has been incompletely known. However, all the spore forms occur upon numbers 547, 773, and 777, and from them the following characters have been obtained to complete the stages.

Pycnia epiphyllous, crowded in small groups, noticeable, subepidermal, globoid, 100–135 μ in diameter.

Aecia chiefly hypophyllous, crowded in small groups of 2 to 8, cylindric, 0.2–0.5 mm. in diameter and about twice as high; peridium with margin somewhat erose, the peridial cells rectangular or rhomboidal, 13–15 by 35–45 μ, abutted, the outer wall 3–4 μ thick, smooth, the inner wall 2–3 μ, verrucose; aeciospores angularly globoid, ellipsoid or oblong, 15–19 by 19–32 μ; wall colorless or pale yellow, 1–2 μ thick, very closely verrucose.

159. PUCCINIA MITRATA Syd. (on Lamiaceae).

Salvia polystachya Orteg., Quezaltenango, Jan. 20, 1915, ii, III, *95*; Solola, 7000 feet alt., Jan. 25, 1915, ii, III, *120*.

Salvia purpurea Cav., San Lucas Toliman, Dept. Solola, Feb. 3, 1915, ii, III, *186*.

Salvia sp., Santa Maria, Dept. Quezaltenango, Jan. 15, 1917, II, III, 724; Colomba, Dept. Quezaltenango, Feb. 3, 1917, II, *825*.

The species occurs also in southern Mexico and Costa Rica. The initial stages in the life cycle are unknown. In the collections on *S. polystachya* and *S. purpurea* here listed, the pore in the lower cell of the

teliospore is in the lower half of the cell, instead of in the usual position close to the septum.

160. PUCCINIA INFREQUENS Holw. (on Lamiaceae).

Salvia cinnabarina Mart. & Gal., San Rafael, Dept. Guatemala, Jan. 7, 1915, ii, III, *19B*; Volcan de Agua, Dept. Sacatépequez, Jan. 13, 1915, II, III, *78*; same, March 4, 1916, II, III, *552*; Quezaltenango, Jan. 21, 1915, II, III, *99*; same, Jan. 16, 1917, II, III, *727*; same, Jan. 18, 1917, II, iii (with *P. delicatula*), *751a*; Antigua, Dept. Sacatépequez, March 2, 1916, II, III, *546*; Huehuetenango, Jan. 23, 1917, II, III, *768*.

The species occurs also in southern Mexico, and has not so far been found on more than the one species of host. The initial stages of the life cycle are unknown.

The species was collected by Kellerman at Volcan de Atitlan, Dept. Solola, Feb. 16, 1906, *5438*, and reported by Kern in Journ. Mycol. *l. c.*

161. **Puccinia** (?) **degener** Mains & Holway sp. nov. (on Lamiaceae).

Salvia albiflora Mart. & Gal. (?), road between Quezaltenango and Colomba, Feb. 4, 1917, II, *838*.

Uredinia hypophyllous, scattered or crowded in small groups, round, 0.1–0.3 mm. in diameter, early naked, pulverulent, cinnamon-brown, ruptured epidermis evident; urediniospores broadly obovoid-globoid, or somewhat flattened from above, 19–23 by 19–25 μ; wall light cinnamon-brown, thin, 1–1.5 μ, moderately and rather prominently echinulate, with one subequatorial pore, usually 5–7 μ from the hilum.

Apparently distinct from all other known Salvia rusts in its single basal pore. Its general resemblance to the preceding group of species makes it seem safe to place the form under the genus Puccinia with no present knowledge of the teliospores.

162. **Puccinia filiola** Mains & Holway sp. nov. (on Lamiaceae).

Salvia involucrata Cav., Solola, Jan. 30, 1915, ii, III, *156* (type).

Salvia pulchella DC., San Rafael, Dept. Guatemala, Jan. 7, 1915, ii, III, *19A*; same, Jan. 9, 1915, II (with *P. delicatula*), *41a*; Totonicapam, Jan. 24, 1915, ii, III, *107*.

Salvia sp., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, III, *579*.

Uredinia hypophyllous, scattered, round, minute, 0.1–0.2 mm. in diameter, early naked, pulverulent, cinnamon-brown, ruptured epi-

dermis inconspicuous; urediniospores oblate-spheroid, 23–27 μ broad by 19–23 μ long, or triangular obovoid, 21–23 μ broad by 23–26 μ long; wall dark cinnamon-brown, 1.5–2 μ thick, moderately and strongly echinulate, the pores 2–3, subequatorial.

Telia among and similar to the uredinia, but somewhat darker; teliospores oblong or ellipsoid, 23–29 by 35–50 μ, rounded at both ends, not constricted at septum; wall chestnut-brown, 2–3.5 μ thick, thickened over the germ pores into a low yellowish umbo, 5–7 μ thick, moderately verrucose with the markings uniting into short irregular lines, giving the appearance of being coarsely verrucose, the pore of lower cell variable; pedicel colorless, two to three times length of spore, with thin wall, 1 μ or less.

The species is closely related to *P. mitrata* Syd., from which it differs in the thinner-walled and more oblong teliospores and in larger and characteristically shaped urediniospores.

A collection made by Lagerheim on *Salvia macrostachya* at Pangor, Ecuador, September, 1891, appears to be the same species, although possessing only uredinia.

This species, taken with the preceding four species, makes up a group of tropical salvia rusts with many intergrading forms. They are to some extent limited by hosts, but morphological characters are at present the chief means of separation. Much of the study of the Salvia rusts has been done by Dr. E. B. Mains, and his discriminating judgment has been followed in assorting the collections. More varied material on well identified hosts is needed to get a clear understanding of this difficult group.

163. PUCCINIA IMPEDITA Mains & Holw. (on Lamiaceae).

Salvia occidentalis Swartz, Antigua, Dept. Sacatépequez, Dec. 27, 1916, II, *642*; San Felipe, Dept. Retalhuleu, Jan. 13, 1916, II, *712*.

The full life cycle for this species is not yet known. The rust is not uncommon in southern Mexico and the West Indies on the same host as well as on others.

164. PUCCINIA DELICATULA (Arth.) Sacc. & Trott. (on Lamiaceae).

Salvia cinnabarina Mart. & Gal., Quezaltenango, Jan. 18, 1917 (with *P. farinacea*), *751*; same, Jan. 23, 1917 (with *P. farinacea*), *769*; same, Jan. 31, 1917, *811*.

Salvia elegans Vahl, Solola, Jan. 28, 1915 (with *P. farinacea*), *140*.

Salvia Holwayi Standley, Quezaltenango, Jan. 18, 1917 (with *P. farinacea*), *743*; Zunil, Dept. Quezaltenango, Jan. 28, 1917, *789*.

Salvia pulchella DC., San Rafael, Dept. Guatemala, Jan. 9, 1915 (with *P. farinacea*), *41*.

Heretofore, this short-cycle, leptoform rust has been known only by a single collection, made by Professor Holway in the Federal District of Mexico. It was published under the name *Polioma delicatula* (Journ. Mycol. **13**: 29. 1907), a genus founded to embrace the short-cycle species of Puccinia having colorless teliospores that germinate in the sorus at maturity. The spores range somewhat shorter in many cases than indicated in the original description and in the North American Flora (**7**: 219).

165. PUCCINIA FIDELIS Arth. (on Lamiaceae).

Hyptis lilacina Schiede & Deppe, San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 7, 1915, II, iii, *27a*.

Hyptis pectinata (L.) Poir. (*Mesosphaerum pectinatum* Kuntze), Solola, Jan. 27, 1915, II, III, *136*.

This long-cycle species with all spore forms has been previously known from Mexico and Guatemala on other species of hosts. It is given in the North American Flora (**7**: 212) under the name *Eriosporangium fidelis* Arth. The Guatemalan collections previously known were made by Kellerman, on *H. urticoides* H.B.K., Laguna, Lake Amatitlan, Jan. 17, 1906, II, iii, *5401*, as well as on *H. lilacina*, Guatemala City, Feb. 1, 1905, II, *5334*, and mentioned by Kern in Journ. Mycol. (**13**: 23. 1907), but not given a name. The urediniospores of this species have basal pores.

166. PUCCINIA MEDELLINENSIS Mayor (on Lamiaceae).

Hyptis pectinata (L.) Poir. (*Mesosphaerum pectinatum* Kuntze), Antigua, 5300 feet alt., Dept. Sacatépequez, Jan. 11, 1915, II, *68*; Guatemala City, Feb. 8, 1916, II, *465*; Aguas Amargas, Dept. Quezaltenango, Jan. 30, 1917, II, *795*.

A long-cycle rust, very common throughout tropical America, especially in the uredinial stage. The species was given in the North American Flora (**7**: 212) under the name *Eriosporangium tucumanense* (*Aecidium tucumanense* Speg.), a name which properly belongs to a South American rust, not yet reported for North America. The description there given applies to *Puccinia medellinensis* Mayor, a species based on a collection from Colombia, S. A., but very common in Central America and the West Indies. The species has urediniospores with two equatorial pores and very similar to those of *P. Hypti-*

dis (Curt.) Trel. & Earle, but with teliospores much shorter. The range is more southernly than that of *P. Hyptidis*.

The entry in the North American Flora (**7**: 212) of *Hyptis spicata* as a host under *Eriosporangium Hyptidis* was founded on four collections made by Kellerman in Guatemala, and reported by Kern (Journ. Myc. **13**: 22) under *Puccinia Hyptidis*. A recent study of these specimens indicates that they should be transferred to *P. medellinensis*, and that the hosts are not *H. spicata*, as reported, but as follows: *H. pectinata* (L.) Poir., Moran, Dept. Amatitlan, Feb. 11, 1905, II, *5310*; *H. polystachya* H.B.K., Moran, Feb. 11, 1905, II, *4327*, *5311*, and Fiscal, Dept. Guatemala, Jan. 11, 1906, II, *5443*.

167. **Puccinia parilis** (Arth.) Arthur comb. nov. (on Lamiaceae).

Hyptis stellulata Benth. (*Mesosphaerum stellulatum* Kuntze), Agua Caliente, Dept. Guatemala, Feb. 10, 1917, O, II, iii, *848*.

This species has heretofore been known only from Mexico and only on *Hyptis pectinata*, a host very similar in its appearance to *H. stellulata*. There are plenty of pycnia scattered over this collection, but rarely associated intimately with the uredinia. The main features of the collection, however, indicate that it should be referred to *P. parilis*, a species without aecidioid aecia, and which has heretofore been called *Argomyces parilis* Arth. (N. Amer. Fl. **7**: 217. 1912).

There is also present on some of the leaves a scanty amount of teliospores that are small, broadly ellipsoid, with a verrucose, dark brown wall and short colorless pedicel. They are much like those of the short-cycle Hyptis rust, *P. distorta* Holw.

168. PUCCINIA PALLIDISSIMA Speg. (on Lamiaceae).

Stachys Lindeni Benth., Agua Amargas, Dept. Quezaltenango, Jan. 30, 1917, *805*.

The species is a short-cycle form. The Sydows (Monog. Ured. **1**: 299. 1902) maintain *Puccinia albida* Diet. & Neg. (Engl. Bot. Jahrb. **24**: 160. 1897), as distinct from *P. pallidissima* on the ground that the latter has the wall of the spore of uniform thickness. Type material has not been seen by the author for either form, but a collection in the Arthur herbarium labeled *P. pallidissima*, made by Lorentz at the type locality of that species in Argentina, and on *Stachys arvensis*, the type host, shows the spores to be thickened above. Believing that the two forms are essentially alike, they are here united. This is the first record of a rust on Stachys for North America.

169. **Puccinia fuscata** Arthur & Holway sp. nov. (on Lamiaceae).

Cunila leucantha Benth., Quezaltenango, Jan. 18, 1917, O, I, II, III, *742*; same, Jan. 28, 1917, O, I, II, III, *785* (type).

Cunila polyantha Benth., Solola, 7000 feet alt., Jan. 31, 1915, O, I, II, iii, *166*.

Pycnia epiphyllous, crowded in small groups, subepidermal, inconspicuous, globoid, 128–150 μ in diameter.

Aecia hypophyllous, in small groups of two to five opposite the pycnia, round, 0.1–0.4 mm. across; peridium wanting and replaced by a more or less definite layer of mycelium; aeciospores ellipsoid or globoid, 23–29 by 26–33 μ; wall colorless, 1.5 μ thick, closely and coarsely verrucose.

Uredinia hypophyllous, scattered, roundish, 0.1–0.6 mm. across, early naked, pulverulent, golden-brown, ruptured epidermis rather inconspicuous; urediniospores broadly ellipsoid or obovoid, 23–26 by 26–32 μ; wall cinnamon-brown, 1.5 μ thick, moderately echinulate, the pores 2, equatorial.

Telia hypophyllous, scattered, round, 0.2–0.4 mm. across, early naked, pulvinate, light chestnut-brown, becoming cinereous by germination, ruptured epidermis inconspicuous; teliospores clavate or oblong, 20–27 by 38–56 μ, rounded above, more or less narrowed below, somewhat constricted at septum; wall dark cinnamon-brown, paler to colorless below, very thin, 1 μ or less, much thicker at apex, 5–10 μ, smooth; pedicel colorless, 35–60 μ long.

An Eriosporangium-like species, having no aecial peridium, and with readily collapsing teliospores that germinate in the sorus at maturity. It differs from *P. Cunilae* Diet. by the broader aeciospores, the presence of uredinia, and the shorter, apically thickened, and more colored teliospores.

170. PUCCINIA NESODES Arth. & Holw. (on Scrophulariaceae).

Castilleja communis Benth., Santa Maria de Jesus, Volcan de Agua, Dept. Sacatépequez, March 4, 1916, *551*; Panajachel, Dept. Solola, Jan. 3, 1917, *673*.

Castilleja tenuiflora Benth., Solola, 5300 feet alt., Jan. 27, 1915, *125* (with *Cronartium coleosporioides* (Diet. & Holw.) Arth.); Antigua, Dept. Sacatépequez, Dec. 28, 1916, *653*.

Castilleja sp., Panajachel, Dept. Solola, Jan. 3, 1917, *669*.

A short-cycle rust occurring also in Costa Rica on species of Lamourouxia.

171. PUCCINIA DEPALLENS Arth. & Holw. (on Bignoniaceae).

Pithecoctenium muricatum DC. (?), Guatemala City, Feb. 15, 1916, O, III, *492*.

A short-cycle rust occurring on the same host in Costa Rica.

172. PUCCINIA RUELLIAE (B. & Br.) Lagerh. (on Acanthaceae).

Justicia sp., San Felipe, Dept. Retalhuleu, Jan. 12, 1917, II, *691*.

The assignment of the collection on Justicia to this species is made without complete certainty, as no telia are present and no previous collection on this host is known. The species is a long-cycle one with all spore forms, and has usually been listed as *P. lateripes* Berk. & Rav.

The same species was collected on *Blechum Brownei* (Swartz) Juss., by Kellerman, at Laguna, Lake Amatitlan, Jan. 17, 1906, II, *5400*, and reported by Kern in Journ. Mycol. *l. c.* and issued in Kellerm. Fungi Sel. Guat. *17*, in both places under the name *P. Tetramerii* Seym. The rust on this host is often called *P. Blechi* Lagerh.

173. **Puccinia varia** (Diet.) Arth. comb. nov. (on Acanthaceae).

Acanthaceae (Ruellia or Jacobina?), Panajachel, Dept. Solola, Jan. 30, 1915, II, iii, *160*.

The uredinia of the collection agree well with those of the type collection for *Uredo varia* Diet. (Hedwigia **36**: 35. 1897), obtained at Rio de Janeiro, Brazil, in December, 1891, E. Ule *1817*. The urediniospores have two equatorial pores. In addition to the uredinia the Guatemalan collection shows a few telia, which are epiphyllous, chestnut-brown, round, 0.3 mm. across, with the ruptured epidermis noticeable. The teliospores are broadly ellipsoid, 24–29 by 39–45 μ, the wall chestnut-brown, 2–3 μ thick, slightly thicker above, 5–6 μ, smooth, with the pedicel tinted and up to 55 μ long. As in the type collection the host remains uncertain, except as to the family.

174. PUCCINIA ELYTRARIAE P. Henn. (on Acanthaceae).

Elytraria (*Tubiflora*) sp., Palin, Dept. Amatitlan, Dec. 24, 1916, *635*.

A short-cycle rust, of which few collections have previously been known, and all from Mexico or Costa Rica.

175. PUCCINIA LATERITIA Berk. & Curt. (on Rubiaceae).

Crucea calocephala DC., Guatemala City, Jan. 8, 1917, *684*.

Spermacoce podocephala DC., Solola, 5100 feet alt., Jan. 27, 1915, *139*; Panajachel, Dept. Solola, Jan. 3, 1917, *662*.

A common short-cycle rust of American tropical regions, extending well north and south into the temperate zones. The first host mentioned is a new one for the rust.

176. **Puccinia eximia** Arthur & Holway sp. nov. (on Rubiaceae).

Galium mexicanum H.B.K. (?), Antigua, Dept. Sacatépequez, March 1, 1916, i, III, *542*.

Galium sp., Volcan de Agua, 7000 feet alt., Dept. Sacatépequez, Jan. 13, 1915, I, ii, III, *81*; Antigua, Dept. Sacatépequez, Dec. 28, 1916, I, III, *645*; Quezaltenango, Jan. 16, 1917, I, II, iii, *735* (type); same, Jan. 31, 1917, I, III, *809*.

Aecia amphigenous, more or less scattered upon rather indefinite yellowish areas, short cylindric, 0.2–0.5 mm. high, 0.2–0.4 mm. in diameter; peridium white, the erose margin soon breaking away, the peridial cells rhombic or rhomboidal, 16–23 by 23–40 μ, slightly overlapping, the outer wall striate, 3–7 μ thick, the inner wall verrucose, 2.5–5 μ thick; aeciospores irregularly globoid or ellipsoid, 18–21 by 19–24 μ; wall colorless, thin, 1–1.5 μ, finely and closely verrucose.

Uredinia hypophyllous, scattered, oval or oblong, 0.6–1 mm. long, somewhat tardily naked, pulverulent, dark cinnamon-brown, ruptured epidermis conspicuous; urediniospores ellipsoid or obovoid, 21–29 by 32–35 μ; wall dark cinnamon-brown, moderately thick, 2–3 μ, coarsely echinulate, the pores 2–3, superequatorial.

Telia hypophyllous, scattered or in small groups, oval, 0.6–0.8 mm. long, early naked, compact, dark chestnut-brown, ruptured epidermis noticeable; teliospores oblong, clavate-oblong, or clavate-ellipsoid, 21–27 by 42–58 μ, round at both ends, or somewhat narrowed below, slightly constricted at septum; wall golden- or pale chestnut-brown, 1.5–2 μ thick, thicker above, 5–12 μ, smooth; pedicel colorless, about as long as the spore.

No pycnia were detected in the collection examined. The species differs from other Galium rusts having uredinia by the large urediniospores with their two or three superequatorial pores. The teliospores are also unusually large. In part of the collections uredinia were absent. When only aecia and telia are present there is a resemblance to *P. ambigua*, but this form differs by the naked telial sorus and lighter-colored teliospores, with their ends more rounded. The teliospores are also noticeably broader, and usually somewhat larger. A eugyrinious species in which the uredinia are slightly or not developed is not unknown, although somewhat rare. The malvaceous rust *Puccinia heterospora*, common in California, not infrequently shows this behavior.

177. PUCCINIA HIERACII (Schum.) Mart. (on Cichoriaceae).

Hieracium sp., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, III, *577*; Antigua, Dept. Sacatépequez, Dec. 28, 1916, II, III, *648*.

A widespread long-cycle rust, having pycnia, uredinia, and telia, found in both temperate and tropical regions.

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ON THE OSMOTIC CONCENTRATION OF THE TISSUE FLUIDS OF PHANEROGAMIC EPIPHYTES[1]

J. ARTHUR HARRIS

INTRODUCTORY REMARKS

The purpose of this paper, which is one of a series dealing with the problem of the physico-chemical properties of vegetable saps in relation to environmental factors and to geographical distribution, is to present the results of three series of determinations of the osmotic concentration of the tissue fluids of phanerogamic epiphytes, and to compare them briefly and in a preliminary way with available data for the osmotic concentrations found in the sap of terrestrial vegetation.

Notwithstanding the enthusiastic interest aroused in the mind of the botanical traveler by the remarkable range of form and the obvious physiological peculiarities of the Orchidaceae, Bromeliaceae, and other epiphytic forms so characteristic of tropical vegetation, our knowledge, in quantitative terms, of the physiology of these organisms is exceedingly meager.

Since I hope on another occasion to discuss epiphytism in greater detail, I shall not in this place review the general literature.

MATERIALS AND METHODS

In this paper I have meant to include only those species which may unquestionably be considered typical epiphytes. It was for this reason that a few determinations made on plants which may be either terrestrial or epiphytic were included by Mr. Lawrence and myself in our paper on the Jamaican montane rain forest vegetation (1917*a*). In some instances it is extremely difficult to determine just which species shall be regarded as epiphytes. Our data are given in detail, and any botanist who chooses may arrange them differently.

The methods employed in the present study are those sufficiently described in our earlier discussion of the parasitic and the terrestrial vegetation of the Blue Mountains (Harris and Lawrence, 1916, 1917*a*).

[1] This study was made possible by the Department of Botanical Research and the Department of Experimental Evolution of the Carnegie Institution of Washington.

The determinations here recorded were secured in three periods of field work, the first in Jamaica in 1915, the second and third in southern Florida in 1916 and 1917. In the first period I had the advantage of the co-operation of Mr. John V. Lawrence, who remained on the island for some time longer than I was able to do, and to whom I am indebted for a large part of the work on Jamaican forms. In the third period Mr. Charles W. Crane rendered most efficient service in several phases of the work.

The determinations were carried out in the Tropical Laboratory at Cinchona, Jamaica, and in the Subtropical Laboratory of the United States Department of Agriculture at Miami, Florida. I have to thank Mr. William Harris, F.L.S., and the members of the British Association Committee for the use of the Laboratory at Cinchona, and am much indebted to Dr. David Fairchild, Agricultural Explorer, and to Mr. Edward Simmonds, in charge of the Plant Introduction Garden at Miami, for the use of the laboratory and other favors. All the species were determined in the herbarium of the New York Botanical Garden. In addition, I am indebted to Dr. Small for various courtesies in the field work.

PRESENTATION OF DATA

The following protocol gives the individual determinations for the several species in terms of freezing point lowering, Δ, corrected for undercooling, and osmotic concentration in atmospheres as determined from a published table (Harris and Gortner, 1914). The averages, designated by bars for each species, are given at the extreme right. When only a single determination is available it has of necessity served to represent the species in place of the average.

In the Bromeliaceae an attempt has been made to arrange the forms in a rough series from the most typical tank forms to those departing most widely from the type in which water storage in the bases of the leaves is possible. Ultimately I hope our determinations will cover a range of forms sufficiently wide and be numerous enough to justify consideration of the problem of the relationship between sap properties and morphological structure in this fascinating family of plants. It has not seemed feasible to attempt any logical classification of the Orchidaceae, and they are merely alphabetically arranged for each of the regions.

All the Jamaican montane rain forest determinations were made in 1915. Hence the year is omitted when dates are cited. In the case of

the Florida determinations, the year as well as the day of the month has been given.

BROMELIACEAE

Guzmannia Sintensii (Baker) Mez $\bar{\Delta} = 0.31, \bar{P} = 3.8$

Montane Rain Forest, Leeward Slopes, Feb. 24, $\Delta = 0.31$, $P = 3.7$; Ridges, Feb. 9, $\Delta = 0.34$, $P = 4.1$; Mar. 9, $\Delta = 0.45$, $P = 5.5$; Jim Crow Peak, Feb. 17, $\Delta = 0.25$, $P = 3.0$; Feb. 17, $\Delta = 0.28$, $P = 3.4$; Windward Slopes and Ravines, Feb. 13, $\Delta = 0.25$, $P = 3.0$; Feb. 13, $\Delta = 0.23$, $P = 2.8$; Feb. 20, $\Delta = 0.28$, $P = 3.3$; Mar. 13, $\Delta = 0.44$, $P = 5.3$.

Guzmannia capituligera (Griseb.) Mez (?) $\bar{\Delta} = 0.44, \bar{P} = 5.2$

Montane Rain Forest, Leeward Slopes, Feb. 18[2], $\Delta = 0.46$, $P = 5.5$; Windward Slopes and Ravines, Feb. 22, $\Delta = 0.41$, $P = 4.9$.

Guzmannia monostachya (L.) Rusby $\bar{\Delta} = 0.46, \bar{P} = 5.6$

Subtropical Florida. Sykes Hammock, Jan. 27, 1916, $\Delta = 0.42$, $P = 5.1$; Mar. 16, 1917, $\Delta = 0.50$, $P = 6.0$.

Catopsis Berteroniana (Schult.) Mez $\bar{\Delta} = 0.46, \bar{P} = 5.6$

Subtropical Florida. Hattie Bauer Hammock, Mar. 19, 1917, $\Delta = 0.46$, $P = 5.5$; Mar. 19, 1917, $\Delta = 0.42$, $P = 5.0$; Royal Palm Hammock, Mar. 3, 1916, $\Delta = 0.50$, $P = 6.0$; Feb. 21, 1917, $\Delta = 0.48$, $P = 5.7$; Small Hammock between Florida City and Biscayne Bay, Feb. 17, 1917, $\Delta = 0.46$, $P = 5.6$.

Tillandsia utriculata L. $\bar{\Delta} = 0.43, \bar{P} = 5.2$

Subtropical Florida. Hattie Bauer Hammock, Mar. 16, 1917, $\Delta = 0.43$, $P = 5.2$; Mar. 19, 1917, $\Delta = 0.45$, $P = 5.4$; Royal Palm Hammock, Mar. 4, 1916, $\Delta = 0.42$, $P = 5.1$; Mar. 3, 1916, $\Delta = 0.33$, $P = 4.0$; Small Hammock near Royal Palm Hammock, Feb. 23, 1917, $\Delta = 0.43$, $P = 5.2$; Bryan Hammock, Feb. 13, 1917, $\Delta = 0.37$, $P = 4.5$; Palm and Live Oak Hammock, Peninsula, near the Narrows, Indian River, Apr. 1, 1917, $\Delta = 0.44$, $P = 5.3$; on dwarfed *Rhizophora Mangle*, near Biscayne Bay, Feb. 17, 1917, $\Delta = 0.40$, $P = 4.8$; Feb. 17, 1917, $\Delta = 0.57$, $P = 6.9$; Feb. 17, 1917, $\Delta = 0.39$, $P = 4.7$; Feb. 17, 1917, $\Delta = 0.34$, $P = 4.1$; Orange Grove, Miami, Feb. 10, 1917, $\Delta = 0.58$, $P = 6.9$.

[2] These determinations are for the older, outer leaves. Sap from the younger leaves gave $\Delta = 0.35$, $P = 4.2$.

Tillandsia Valenzuelana A. Rich. $\overline{\Delta} = 0.36$, $\overline{P} = 4.3$

Subtropical Florida. Royal Palm Hammock, Jan. 29, 1916, $\Delta = 0.35$, $P = 4.2$; Jan. 29, 1916, $\Delta = 0.36$, $P = 4.3$; Feb. 23, 1917, $\Delta = 0.34$, $P = 4.0$; Small Hammock near Royal Palm Hammock, Feb. 23, 1917, $\Delta = 0.41$, $P = 4.9$; Bryan Hammock, Feb. 13, 1917, $\Delta = 0.32$, $P = 3.9$; Brickell Hammock, Mar. 9, 1917, $\Delta = 0.38$, $P = 4.5$.

Tillandsia incurva Griseb. (?) $\overline{\Delta} = 0.25$, $\overline{P} = 3.0$

Montane Rain Forest, Ridges, Feb. 18, $\Delta = 0.28$, $P = 3.3$; Windward Slopes and Ravines, Mar. 2, $\Delta = 0.22$, $P = 2.7$.

Tillandsia fasciculata Swartz $\overline{\Delta} = 0.39$, $\overline{P} = 4.6$

Subtropical Florida. Hattie Bauer Hammock, Mar. 16, 1917, $\Delta = 0.36$, $P = 4.3$; Mar. 19, 1917, $\Delta = 0.33$, $P = 3.9$; Royal Palm Hammock, Mar. 4, 1916, $\Delta = 0.40$, $P = 4.8$; Feb. 27, 1917, $\Delta = 0.41$, $P = 5.0$; Small Pineland Hammock, near Royal Palm Hammock, Mar. 4, 1916, $\Delta = 0.44$, $P = 5.3$; Sykes Hammock, Jan. 27, 1916, $\Delta = 0.40$, $P = 4.8$; Bryan Hammock, Feb. 13, 1917, $\Delta = 0.31$, $P = 3.7$; Small Hammock near Royal Palm Hammock, Feb. 23, 1917, $\Delta = 0.43$, $P = 5.2$; Murden Hammock, Jan. 28, 1916, $\Delta = 0.45$, $P = 5.4$; Small Hammock between Biscayne Bay and Florida City, Feb. 17, 1917, $\Delta = 0.32$, $P = 3.9$.

Tillandsia aloifolia Hook.

Subtropical Florida. On dwarfed *Rhizophora Mangle* near Biscayne Bay, Feb. 17, 1917, $\Delta = 0.43$, $P = 5.1$.

Tillandsia Balbisiana Schult. $\overline{\Delta} = 0.46$, $\overline{P} = 5.5$

Subtropical Florida. Hattie Bauer Hammock, Mar. 19, 1917, $\Delta = 0.53$, $P = 6.4$; Royal Palm Hammock, Jan. 29, 1916, $\Delta = 0.40$, $P = 4.8$; Mar. 3, 1916, $\Delta = 0.39$, $P = 4.7$; Feb. 23, 1917, $\Delta = 0.47$, $P = 5.7$; Small Hammock near Royal Palm Hammock, Feb. 23, 1917, $\Delta = 0.49$, $P = 6.0$; Bryan Hammock, Feb. 13, 1917, $\Delta = 0.38$, $P = 4.6$; on dwarfed *Rhizophora Mangle* near Biscayne Bay, Feb. 29, 1916, $\Delta = 0.50$, $P = 6.0$; Feb. 17, 1917, $\Delta = 0.51$, $P = 6.1$.

Tillandsia tenuifolia L. $\overline{\Delta} = 0.42$, $\overline{P} = 5.1$

Subtropical Florida. Royal Palm Hammock, Jan. 29, 1916, $\Delta = 0.38$, $P = 4.6$; Feb. 21, 1917, $\Delta = 0.45$, $P = 5.4$; Sykes Hammock, Jan. 27, 1916, $\Delta = 0.46$, $P = 5.5$; Mar. 15, 1917, $\Delta = 0.45$, $P = 5.5$; Bryan Hammock, Feb. 13, 1917, $\Delta = 0.37$, $P = 4.4$.

Tillandsia recurvata L. $\bar{\Delta} = 0.49$, $\bar{P} = 5.8$

Subtropical Florida. Royal Palm Hammock, Mar. 3, 1916, $\Delta = 0.52$, $P = 6.2$; Feb. 23, 1917, $\Delta = 0.45$, $P = 5.4$.

Dendropogon usneoides (L.) Raf. $\bar{\Delta} = 0.75$, $\bar{P} = 9.0$

Subtropical Florida. Hattie Bauer Hammock, Mar. 19, 1917, $\Delta = 0.62$, $P = 7.5$; Royal Palm Hammock, Mar. 3, 1916, $\Delta = 0.86$, $P = 10.4$; Bryan Hammock, Feb. 13, 1917, $\Delta = 0.50$, $P = 6.0$; Palm and Live Oak Hammock, the Peninsula near the Narrows, Indian River, Apr. 1, 1917, $\Delta = 1.32$, $P = 15.8$; Sykes Hammock, Mar. 15, 1917, $\Delta = 0.57$, $P = 6.8$; Orange Grove, Miami, Mar. 6, 1916, $\Delta = 0.70$, $P = 8.4$; Feb. 9, 1917, $\Delta = 0.65$, $P = 7.9$.

ORCHIDACEAE

Epidendrum imbricatum Lindl.

Montane Rain Forest, Windward Slopes and Ravines, Feb. 13, $\Delta = 0.30$, $P = 3.6$.

Lepanthes ovalis (Swartz) Fawc. & Rendle

Montane Rain Forest, Leeward Ravines, Mar. 18, $\Delta = 0.35$, $P = 4.2$.

Lepanthes divaricata Fawc. & Rendle $\bar{\Delta} = 0.20$, $\bar{P} = 2.4$

Montane Rain Forest, Ridges, Feb. 9, $\Delta = 0.19$, $P = 2.3$; Mar. 9, $\Delta = 0.27$, $P = 3.3$; Jim Crow Peak, Feb. 17, $\Delta = 0.16$, $P = 1.9$; Windward Slopes and Ravines, Feb. 20, $\Delta = 0.19$, $P = 2.3$; Feb. 24, $\Delta = 0.18$, $P = 2.2$; Mar. 4, $\Delta = 0.20$, $P = 2.4$.

Octadesmia montana (Swartz) Benth. $\bar{\Delta} = 0.44$, $\bar{P} = 5.3$

Montane Rain Forest, Jim Crow Peak, Feb. 17, $\Delta = 0.41$, $P = 5.0$; Windward Slopes and Ravines, Feb. 20, $\Delta = 0.46$, $P = 5.5$.

Pleurothallis racemiflora (Swartz) Lindl.

Montane Rain Forest, Leeward Ravines, Mar. 11, $\Delta = 0.21$, $P = 2.6$.

Stelis micrantha Swartz $\bar{\Delta} = 0.22$, $\bar{P} = 2.6$

Montane Rain Forest, Ridges, Feb. 9, $\Delta = 0.23$, $P = 2.7$; Windward Slopes and Ravines, Feb. 4, $\Delta = 0.24$, $P = 2.9$; Feb. 13, $\Delta = 0.20$, $P = 2.4$; Feb. 20, $\Delta = 0.21$, $P = 2.5$.

Stelis ophioglossoides Swartz

Montane Rain Forest, Jim Crow Peak, Feb. 17, $\Delta = 0.22$, $P = 2.7$.

Anacheilium cochleatum (L.) Hoffmannsegg $\overline{\Delta} = 0.43$, $\overline{P} = 5.2$

Subtropical Florida. Hattie Bauer Hammock, Jan. 28, 1916, $\Delta = 0.44$, $P = 5.3$; Royal Palm Hammock, Jan. 29, 1916, $\Delta = 0.41$, $P = 5.0$.

Auliza nocturna (L.) Small $\overline{\Delta} = 0.42$, $\overline{P} = 5.0$

Subtropical Florida. Hattie Bauer Hammock, Jan. 28, 1916, $\Delta = 0.46$, $P = 5.5$; Mar. 16, 1917, $\Delta = 0.47$, $P = 5.7$; Mar. 19, 1917, $\Delta = 0.52$, $P = 6.3$; Royal Palm Hammock, Jan. 29, 1916, $\Delta = 0.38$, $P = 4.5$; Feb. 21, 1917, $\Delta = 0.29$, $P = 3.5$; Small Pineland Hammock, near Royal Palm Hammock, Mar. 3, 1916, $\Delta = 0.41$, $P = 4.9$; Bryan Hammock, Feb. 13, 1917, $\Delta = 0.40$, $P = 4.8$; Feb. 13, 1917, $\Delta = 0.39$, $P = 4.6$.

Encyclia tampense (Lindl.) Small $\overline{\Delta} = 0.48$, $\overline{P} = 5.8$

Subtropical Florida. Hattie Bauer Hammock, Jan. 28, 1916, $\Delta = 0.48$, $P = 5.8$; Mar. 16, 1917, $\Delta = 0.50$, $P = 6.1$; Mar. 19, 1917, $\Delta = 0.51$, $P = 6.2$; Royal Palm Hammock, Jan. 29, 1916, $\Delta = 0.44$, $P = 5.3$; Brickell Hammock, Mar. 22, 1917, $\Delta = 0.49$, $P = 5.9$; Mar. 24, 1917, $\Delta = 0.40$, $P = 4.8$; Bryan Hammock, Feb. 13, 1917, $\Delta = 0.45$, $P = 5.4$; Palm and Live Oak Hammock, Peninsula, near the Narrows, Indian River, Apr. 1, 1917, $\Delta = 0.62$, $P = 7.4$; Small Hammock near Royal Palm Hammock, Feb. 23, 1917, $\Delta = 0.47$, $P = 5.6$.

Macradenia lutescens R. Br. $\overline{\Delta} = 0.51$, $\overline{P} = 6.1$

Subtropical Florida. Royal Palm Hammock, Jan. 29, 1916, $\Delta = 0.53$, $P = 6.4$; Feb. 21, 1917, $\Delta = 0.48$, $P = 5.7$.

Polystachya minuta (Aubl.) Britton

Subtropical Florida. Bryan Hammock, Feb. 13, 1917, $\Delta = 0.50$, $P = 6.0$.[3]

Spathiger rigidus (Jacq.) Small $\overline{\Delta} = 0.38$, $\overline{P} = 4.5$

Subtropical Florida. Hattie Bauer Hammock, Jan. 28, 1916, $\Delta = 0.47$, $P = 5.7$; Mar. 16, 1917, $\Delta = 0.36$, $P = 4.4$; Mar. 19, 1917, $\Delta = 0.42$, $P = 5.0$; Royal Palm Hammock, Jan. 29, 1916, $\Delta = 0.35$,

[3] Sample from Hattie Bauer Hammock obtained January 28, 1916, and one from the Brickell Hammock, March 22, 1917, were so mucilaginous that no determination could be made. The juice of the sample from the Bryan Hammock was also highly mucilaginous and could not be filtered. Until verification this determination must be taken as only approximate.

$P = 4.2$; Jan. 29, 1916, $\Delta = 0.31$, $P = 3.7$; Mar. 3, 1916, $\Delta = 0.40$, $P = 4.8$; Feb. 21, 1917, $\Delta = 0.32$, $P = 3.9$.

Vanilla Eggersii Rolfe

Subtropical Florida. Brickell Hammock, Feb. 14, 1916, $\Delta = 0.24$, $P = 2.9$. This determination is of course based on sap from the stems.

PIPERACEAE

Peperomia basellifolia H.B.K. $\overline{\Delta} = 0.35$, $\overline{P} = 4.2$

Montane Rain Forest, Windward Slopes and Ravines, Feb. 20, $\Delta = 0.40$, $P = 4.8$; Feb. 24, $\Delta = 0.35$, $P = 4.2$; Mar. 4, $\Delta = 0.33$, $P = 3.9$; Mar. 13, $\Delta = 0.31$, $P = 3.7$.

Peperomia crassicaulis Fawc. & Rendle $\overline{\Delta} = 0.40$, $\overline{P} = 4.9$

Montane Rain Forest, Ridges, Feb. 18, $\Delta = 0.40$, $P = 4.8$; Mar. 9, $\Delta = 0.44$, $P = 5.2$; Mar. 13, $\Delta = 0.42$, $P = 5.1$; Mar. 16, $\Delta = 0.46$, $P = 5.6$; Windward Slopes and Ravines, Mar. 4, $\Delta = 0.30$, $P = 3.6$.

Peperomia magnoliafolia (Jacq.) A. Dietr. $\overline{\Delta} = 0.38$, $\overline{P} = 4.6$

Subtropical Florida, Royal Palm Hammock, Jan. 29, 1916, $\Delta = 0.38$, $P = 4.6$; Small Hammock near Royal Palm Hammock, Feb. 23, 1917, $\Delta = 0.39$, $P = 4.7$; Bryan Hammock, Feb. 13, 1917, $\Delta = 0.37$, $P = 4.5$; Feb. 13, 1917, $\Delta = 0.35$, $P = 4.2$; Sykes Hammock, Jan. 27, 1916, $\Delta = 0.41$, $P = 4.9$.

Peperomia Myrtillus Miquel $\overline{\Delta} = 0.36$, $\overline{P} = 4.3$

Montane Rain Forest, Leeward Ravines, Mar. 11, $\Delta = 0.35$, $P = 4.2$; Windward Slopes and Ravines, Mar. 13, $\Delta = 0.36$, $P = 4.4$.

Peperomia quadrifolia (L.) H.B.K.

Montane Rain Forest, Leeward Ravines, Mar. 11, $\Delta = 0.39$, $P = 4.6$.

Peperomia septemnervis Ruiz & Pav. $\overline{\Delta} = 0.31$, $\overline{P} = 3.7$

Montane Rain Forest, Leeward Ravines, Mar. 11, $\Delta = 0.33$, $P = 3.9$; Windward Slopes and Ravines, Feb. 13, $\Delta = 0.31$, $P = 3.7$; Feb. 13, $\Delta = 0.30$, $P = 3.6$.

GESNERACEAE

Columnea hirsuta Swartz $\overline{\Delta} = 0.36$, $\overline{P} = 4.3$

Montane Rain Forest, Leeward Ravines, Feb. 26, $\Delta = 0.40$, $P = 4.8$; Windward Slopes and Ravines, Feb. 13, $\Delta = 0.33$, $P = 4.0$; Feb. 20, $\Delta = 0.34$, $P = 4.1$; Feb. 22, $\Delta = 0.33$, $P = 4.0$; Feb .22,

$\Delta = 0.32$, $P = 3.9$; Feb. 24, $\Delta = 0.36$, $P = 4.4$; Mar. 2, $\Delta = 0.37$, $P = 4.5$; Mar. 4, $\Delta = 0.38$, $P = 4.5$; Mar. 13, $\Delta = 0.40$, $P = 4.8$.

ANALYSIS OF DATA

In this paper I shall limit discussion of the data presented to a comparison of the constants of the epiphytes among themselves and with the values which have already been obtained for terrestrial forms in various habitats. Even these comparisons must be limited by the still unorganized condition of our data for several important habitats. Since, however, it will be many months before all of these data can be fully analyzed and ready for discussion, it has seemed proper to place the data which have been obtained for epiphytes during the past three years on record in a form which will enable other physiologists and phytogeographers to use them.

Consider first of all the relative magnitudes of the osmotic concentrations found in the epiphytic plants of the two regions considered. The results, grouped by families, are shown in table 1.

The constants in this table are the averages of species means, not of species determinations (except when only one determination is available for a species), for each family. While the species means which are based upon a large number of determinations are somewhat more trustworthy than those which are based upon only two or three readings, or upon only a single collection, the general mean for the habitat is certainly more representative when calculated in this way than if the habitat average had been computed directly from the individual constants, thus weighting the species with the numbers of collections of each which happened to be made.

TABLE 1

Comparison of Osmotic Concentrations in Jamaican and Floridian Epiphytes

	Jamaica	Florida	Difference
Bromeliaceae	$\bar{\Delta} = 0.333$, $\bar{P} = 4.00$ 2 genera, 3 species	$\bar{\Delta} = 0.464$, $\bar{P} = 5.57$ 4 genera, 10 species	$\bar{\Delta} = +0.131$, $\bar{P} = +1.57$
Orchidaceae	$\bar{\Delta} = 0.276$, $\bar{P} = 3.32$ 5 genera, 7 species	$\bar{\Delta} = 0.421$, $\bar{P} = 5.06$ 7 genera, 7 species	$\bar{\Delta} = +0.145$, $\bar{P} = +1.74$
Piperaceae	$\bar{\Delta} = 0.362$, $\bar{P} = 4.34$ Peperomia only, 5 species	$\bar{\Delta} = 0.380$, $\bar{P} = 4.58$ *Peperomia magnoliaefolia* only	$\bar{\Delta} = +0.018$, $\bar{P} = +0.24$
Gesneraceae	$\bar{\Delta} = 0.358$, $\bar{P} = 4.33$ *Columnea hirsuta* only	——— No representative.	———

The table brings out clearly two facts:

1. That in all four families and in both Jamaica and Florida, the osmotic concentration of epiphytic forms is extremely low.

2. That for the three groups represented in both regions the osmotic concentration of the epiphytes (chiefly from the hammocks) of subtropical Florida is higher than that demonstrated in the Jamaican rain forest. The average difference is 1.57 atmospheres higher for the Bromeliaceae,[4] 1.74 atmospheres higher for the Orchidaceae, and 0.24 atmospheres higher for the single species of *Peperomia*.

The comparison may be made somewhat more analytically on the basis of the means for the genera.

The constants in table 2 are averages of the species means of each of the genera.

TABLE 2

Genera of Jamaican and Floridian Epiphytes Arranged in the Order of the Average Osmotic Concentration of Their Species

Jamaica		Florida	
Genus	$\bar{P}$	$\bar{P}$	Genus
Pleurothallis	2.57		
Stelis	2.65		
		2.90	Vanilla
Tillandsia	3.00		
Lepanthes	3.28		
Epidendrum	3.56		
Columnea	4.33		
Peperomia	4.34		
Guzmannia	4.49		
		4.52	Spathiger
		4.58	Peperomia
		4.97	Auliza
		5.09	Tillandsia
		5.15	Anacheilium
Octadesmia	5.25		
		5.55	Guzmannia
		5.56	Catopsis
		5.83	Encyclia
		6.00	Polystachya
		6.05	Macradenia
		8.97	Dendropogon

[4] That the higher value for Floridian Bromeliaceae is not primarily due to the inclusion of *Dendropogon usneoides* (= *Tillandsia usneoides*) is shown by the fact that if this species be omitted from the Florida series, the remaining 9 species average $\bar{\Delta} = 0.433$, $\bar{P} = 5.19$, which are respectively 0.100 and 1.19 greater than the Jamaican average.

It is clear at a glance that with the exception of the stem-succulent Vanilla in the Floridian and of *Octadesmia montana* in the Jamaican constants, the two series do not overlap in the average (generic) magnitude of their constants. With the exceptions noted, the Jamaican (rain forest) genera range from 2.57 to 4.49 atmospheres, whereas the Floridian genera range from 4.52 to 8.97 atmospheres.

Instead of limiting our comparisons between the two regions to means, the individual determinations may be seriated according to their magnitude and the frequency distributions compared. This has the advantage of giving a general view of the range of variation in the individual constants, but the disadvantage from the standpoint of exact comparison that certain species are far more extensively represented than others. The frequency distributions are given in table 3.

TABLE 3

Frequency Distributions of Osmotic Concentration Determinations in Jamaican and Floridian Epiphytes

Osmotic Concentration in Atmospheres	Orchidaceae		Bromeliaceae	
	Jamaica	Florida	Jamaica	Florida
1.5–1.9	1	—	—	—
2.0–2.4	5	—	—	—
2.5–2.9	5	1	2	—
3.0–3.4	1	—	5	—
3.5–3.9	1	3	1	4
4.0–4.4	1	2	1	7
4.5–4.9	—	6	1	11
5.0–5.4	1	5	1	14
5.5–5.9	1	7	2	6
6.0–6.4	—	5	—	7
6.5–6.9	—	—	—	2
7.0–7.4	—	1	—	—
	16	30	13	51

Because of the unusually high values found in the Spanish moss (*Dendropogon usneoides*) it has been omitted from this table. Notwithstanding this fact, the Floridian Bromeliaceae as well as the Orchidaceae show distinctly higher minima and maxima than the Jamaican forms. The distinction between the two regions is not as clearly shown by the distribution of the individual determinations as by the generic means, since individual determinations must be expected to show much wider variation than averages.

I now turn to the relative magnitude of the osmotic concentration of terrestrial and epiphytic plants.

Since in a number of series of determinations we have found a differentiation in the sap properties of ligneous and herbaceous plants,[5] I shall compare epiphytic Orchidaceae, Bromeliaceae, and Piperaceae primarily with terrestrial herbaceous plants.

Unfortunately the several hundreds of determinations from the various coastal, pineland, hammock, and Everglade habitats of Subtropical Florida are as yet unclassified, and it will probably require some time before the results from this highly interesting region are discussed in detail.

The averages for the various groups of epiphytes from Jamaica and from Subtropical Florida have been given in table 1.

The average freezing-point lowering of the saps ranges from 0.276° to 0.464°, less than two tenths of one degree. In terms of osmotic concentration the values lie between 3.3 and 5.6 atmospheres, a range of less than two and one third atmospheres.

The only extensive series of averages for herbaceous terrestrial vegetation are those for the Arizona deserts made by Harris, Lawrence, and Gortner (1916), and the first Long Island series, by Harris, Lawrence, and Gortner, as yet unpublished, and the Jamaican montane rain forest series which will be treated in greater detail below.

For the Long Island habitats the preliminary average values are:

Habitat	Average Concentration, $\overline{P}$
Beaches, coastal sand dunes, and marshes	13.62
Dryer woods and open fields	10.04
Permanently moist localities	9.27
All habitats	10.41

Note that the epiphytic forms show a sap concentration about one third to one half as great.

For the Arizona desert (vernal) flora the averages for herbaceous plants are:

[5] For averages for divers growth forms from the Arizona deserts see Harris, Lawrence, and Gortner (1916). Averages for Long Island and Jamaican habitats are given by Harris and Lawrence (1917*a*). Some general comparisons are made by Harris (1917).

Habitat	Average Concentration, $\bar{P}$
Rocky slopes	15.94
Canyons	13.33
Arroyos	12.99
Bajada slopes	20.53
Salt spots	23.57
All habitats	15.15

These values are (roughly speaking) from 4 to 7 times as large as those for the epiphytic families.

For the Jamaican series, and unfortunately only for the Jamaican series, it is possible at this time to compare the averages for epiphytic and terrestrial forms from the same habitat.

Table 4 gives the averages of the species means for each habitat for the Orchidaceae and Bromeliaceae and for the genus Peperomia of

TABLE 4

Comparison of Osmotic Concentration of Epiphytic Plants with that of Terrestrial Herbaceous Plants in the Montane Rain Forest

Habitats	Average for Terrestrial Herbs	Orchidaceae		Bromeliaceae		Piperaceae	
		Average for Epiphytic Orchidaceae	Difference and Relative Value	Average for Epiphytic Bromeliaceae	Difference and Relative Value	Average for Epiphytic Piperaceae	Difference and Relative Value
Ruinate of the leeward slopes	$\bar{\Delta}=0.812$ $\bar{P}=9.77$ $(n=17)$	—	—	$\bar{\Delta}=0.385$ $\bar{P}=4.60$ $(n=2)$	-0.427 -5.17 47.1%	—	—
Leeward ravines	$\bar{\Delta}=0.628$ $\bar{P}=7.59$ $(n=13)$	$\bar{\Delta}=0.280$ $\bar{P}=3.40$ $(n=2)$	-0.348 -4.19 44.8%	—	—	$\bar{\Delta}=0.357$ $\bar{P}=4.23$ $(n=3)$	-0.271 -3.36 55.7%
Ridges and peaks	$\bar{\Delta}=0.718$ $\bar{P}=8.63$ $(n=8)$	$\bar{\Delta}=0.267$ $\bar{P}=3.22$ $(n=4)$	-0.451 -5.41 37.3%	$\bar{\Delta}=0.305$ $\bar{P}=3.65$ $(n=2)$	-0.413 -4.98 42.3%	$\bar{\Delta}=0.431$ $\bar{P}=5.19$ $(n=1)$	-0.287 $-3\ 44$ 60.1%
Windward slopes and ravines	$\bar{\Delta}=0.627$ $\bar{P}=7.52$ $(n=15)$	$\bar{\Delta}=0.292$ $\bar{P}=3.50$ $(n=4)$	-0.335 -4.02 46.5%	$\bar{\Delta}=0.310$ $\bar{P}=3.73$ $(n=3)$	-0.317 -3.79 49.6%	$\bar{\Delta}=0.330$ $\bar{P}=3.98$ $(n=4)$	-0.297 -3.54 52.9%
All habitats	$\bar{\Delta}=0.700$ $\bar{P}=8.43$ $(n=53)$	$\bar{\Delta}=0.280$ $\bar{P}=3.37$ $(n=10)$	-0.420 -5.06 40.0%	$\bar{\Delta}=0.330$ $\bar{P}=3.96$ $(n=7)$	-0.370 -4.47 47.0%	$\bar{\Delta}=0.353$ $\bar{P}=4.23$ $(n=8)$	-0.347 -4.20 50.2%

the Piperaceae. The number under each of the averages is the number of species, not the number of determinations, upon which it is based.

The averages for terrestrial herbaceous species are those already published (Harris and Lawrence, 1917*a*).

The general mean for the region has been computed by averaging the species means for the individual habitats. Thus if a species occurs in both the Leeward Ravines and the Ridge Forest it is counted twice, whereas the species which occur in one of these habitats only will be counted but once. Thus the numbers of the species given for all habitats is the number of species weighted with the number of the sub-habitats in which they occur.[6]

The comparison between the epiphytic and the terrestrial herbaceous forms has been made in two ways. First, the actual differences in the average depression of the freezing point and in the average calculated osmotic concentration have been determined and are given with their signs. Second, the average values of P of the epiphytes have been expressed as a percentage of the value for terrestrial herbs.[7]

An examination of the nine comparisons between the epiphytic and terrestrial herbs of the four individual habitats shows that the concentration is in every instance lower for the epiphytic forms. The averages are roughly 4.0 to 5.4 atmospheres lower in the Orchidaceae, 3.8 to 5.2 atmospheres lower in the Bromeliaceae, and 3.4 to 3.6 atmospheres lower in Peperomia of the Piperaceae.[8]

There now remains for consideration only the half shrubby gesneraceous epiphyte *Columnea hirsuta*. One determination from the Leeward Ravines gives $\Delta = 0.395$, $P = 4.76$. Eight constants from the Windward Slopes and Ravine average $\overline{\Delta} = 0.354$, $\overline{P} = 4.28$. If these be compared with the averages for herbaceous vegetation from the same habitats, differences in P of -2.83 for the Leeward Ravine determination and of -3.24 for the Windward habitats are secured.

[6] This method of computing the average has both advantages and disadvantages. For present purposes it is quite adequate.

[7] Practically the same percentages are secured by using the average values of freezing-point lowering, but since the relationship between Δ and P is not strictly linear the results are not exactly identical.

[8] Comparisons with the herbaceous plants of the regions as a whole show a concentration 5.1 atmospheres lower for Orchidaceae, 4.5 atmospheres lower for Bromeliaceae, and 4.2 atmospheres lower for Peperomia of the Piperaceae. The averages for the whole region is obtained by weighting those of the individual habitats with the number of species examined.

If the comparison be made with the ligneous terrestrial vegetation, the differences are −6.07 for the Leeward and −5.45 for the Windward habitats.

In relative terms, the osmotic concentrations of the sap of the epiphytic Orchidaceae is only 37.3 to 46.5 percent as high as that of the terrestrial herbs of the same habitat, the constants for the Bromeliaceae range from 42.3 to 49.6 percent of the comparable values for terrestrial herbs, while the determinations based on Peperomia range from 52.9 to 60.1 percent of those for the non-epiphytic herbs of the same habitats. Columnea shows a concentration of 56.9 percent of that of herbaceous plants in the Windward habitats and 62.7 percent of that of herbaceous plants in the Leeward habitats. If compared with ligneous terrestrial vegetation it shows a concentration of 44.0 percent in the Windward and of 44.0 percent in the Leeward habits.

Summarizing the results of this comparison: the osmotic concentration of the fluids of the epiphytic Orchidaceae, Bromeliaceae, Piperaceae, and Gesneraceae of the montane rain forest of Jamaica is roughly speaking only 37.3 to 62.7 percent as high as that of the terrestrial plants of the same region.

The averages for herbaceous forms include, as already explained, a few determinations based on species which may occur on the ground or as epiphytes. They also include those based on a few ferns and fern allies. The removal of these constants might change *slightly* the actual values of the difference in the table. Since the forms which have been classified as terrestrial but may occur as epiphytes are characterized by lower osmotic concentration than the vegetation as a whole, the removal of these species from the list of herbaceous plants would make the differences demonstrated between terrestrial and epiphytic vegetation even larger. The exclusion of the few determinations for terrestrial ferns and fern allies could be justified only on the assumption that they are sensibly differentiated in their sap properties from flowering plants. There is, at present, no basis for such an assumption.

The low concentration of the sap of epiphytic Phanerogams may perhaps be most clearly brought out by comparing it with that of the ligneous species upon which they may occur. Table 5 gives the differences and relative concentrations for the Jamaican materials.

Epiphytic Orchidaceae show from 28 to 36 percent, the epiphytic Bromeliaceae from 32 to 38 percent, the epiphytic Piperaceae from 39 to 45 percent, and the epiphytic Gesneraceae about 44 percent

of the osmotic concentration exhibited by the foliage of the ligneous forms upon which they find lodgment. Of course these figures are only approximations, which will be somewhat modified by further work, but they are based on sufficient data to justify the conclusion that the epiphytic species of the rain forest are characterized by a concentration of about one third to one half that of the ligneous terrestrial species.

TABLE 5

Comparison of Osmotic Concentration of Epiphytic Forms with that of Ligneous Terrestrial Species in the Montane Rain Forest

Habitats	Average for Ligneous Plants	Difference and Relative Value			
		Orchidaceae	Bromeliaceae	Piperaceae	Gesneraceae
Ruinate of the leeward slopes...	13.05 ($n=40$)	—	−8.45 35.2%	—	—
Leeward ravines.....	10.83 ($n=32$)	−7.43 31.4%	—	−6.60 39.1%	−6.07 44.0%
Ridges and peaks	11.54 ($n=36$)	−8.32 27.9%	−7.89 31.6%	−6.35 45.0%	—
Windward slopes and ravines...	9.73 ($n=28$)	−6.23 36.0%	−6.00 38.3%	−5.75 40.9%	−5.45 44.0%
All habitats.....	11.44 ($n=136$)	−8.07 29.5%	−7.48 34.6%	−7.21 37.0%	−7.11 37.8%

In passing it may be worth while to point out that these results have an important bearing upon theories of the origin of parasitism. The suggestion has been made that epiphytism is the first stage in the evolution of parasitism in the flowering plants. But all of these most typical epiphytes are characterized by very low osmotic concentration in comparison with the ligneous species of the same region, whereas the Loranthaceae of these forests have been shown (Harris and Lawrence, 1916) to have generally higher concentration of their tissue fluids than their hosts. Similar relationships have been found to exist in desert Loranthaceae (Harris, 1918).

Theoretically one of the best methods of comparison would be to lay side by side constants for terrestrial and epiphytic members of the same family. Unfortunately I have not been able to secure terrestrial Orchidaceae from subtropical Florida. Determinations have been published (Harris and Lawrence, 1917*a*) for Jamaican species. *Epidendrum verrucosum*, which we included in our first paper because we always found it growing on the ground, although Fawcett and Rendle

record it as occurring "on trees, rocks, and dry banks," gives on the average $\overline{\Delta} = 0.51$, $\overline{P} = 6.1$ in the Leeward ravines and $\overline{\Delta} = 0.55$, $\overline{P} = 6.6$ in the ruinate. *Prescottia stachyoides* from the windward ravines and slopes gave an average depression of $\overline{\Delta} = 0.52$, or an average concentration in atmospheres of $\overline{P} = 6.3$.

All of these values are distinctly, and in many cases very much, greater than those obtained from the individual species of epiphytic Orchidaceae.

For comparison with the epiphytic Peperomia we have only *Peperomia stellata*, which we collected in Jamaica only as a terrestrial herb. It gave the following values:

Leeward ravines, $\overline{\Delta} = 0.43$, $\overline{P} = 5.2$
Ridge Forest, $\overline{\Delta} = 0.45$, $\overline{P} = 5.4$
Leeward habitats, $\overline{\Delta} = 0.42$, $\overline{P} = 5.1$

These values are slightly higher than the averages for any of the epiphytic species from the rain forest.

As far as I am aware, the only determination of osmotic concentration of the tissue fluids of any bromeliad hitherto made is that for *Bromelio Pinguim*, which Mr. Lawrence and I (1917*b*) found growing as a terrestrial plant in the Jamaican coastal deserts. This gave $\Delta = 0.63$, $P = 7.6$. This is a value higher than any of those recorded in this paper with the exception of those for *Dendropogon usneoides*. It is, however, extremely low for such a habitat as the Jamaican Coastal Deserts.

With regard to two species which Mr. Lawrence and I treated with the terrestrial vegetation but which others have observed growing as air plants, the following points may be noted.

The woody-stemmed *Blakea trinervia*, which may be rooted in the soil or, according to Shreve, grown as an epiphyte, has a concentration measured by $\overline{\Delta} = 0.58$, $\overline{P} = 6.9$, as compared with the general average of $\overline{\Delta} = 0.81$, $\overline{P} = 9.7$ for the ligneous species of the windward habitats in which it occurs.

Tradescantia multiflora, which we included with terrestrial vegetation in our earlier paper, but which may also occur as an epiphyte, gave in a single determination $\Delta = 0.39$, $P = 4.7$. This is far lower than the general averages of $\overline{\Delta} = 6.3$, $\overline{P} = 7.6$ for the herbs of the Leeward ravines.

CONCLUSIONS

The osmotic concentration of the tissue fluids of epiphytic Bromeliaceae, Orchidaceae, Piperaceae, and Gesneraceae is far lower than that of terrestrial vegetation.

In the Jamaican montane rain forest where direct comparisons for individual habitats are possible, the epiphytes show from 37 to 60 percent of the concentration characteristic of herbaceous terrestrial vegetation, and from 28 to 45 percent of the concentration of ligneous terrestrial vegetation.

In the Bromeliaceae, Orchidaceae, and Peperomia of the Piperaceae, the osmotic concentration of the species of the Jamaican montane rain forest is lower than that of the species of the hammocks of subtropical Florida.

At some future time I hope to deal with the problem of the osmotic concentration of cryptogamic epiphytes and to obtain data on the inorganic and organic constituents of the fluids of epiphytes which will justify further discussion of the physiology of these ecologically remarkable forms.

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ENDURANCE OF EXTREME CONDITIONS AND ITS RELATION TO THE THEORY OF ADAPTATION

W. J. V. OSTERHOUT

Current theories lead us to expect the adaptation of plants to extreme drought or to extreme moisture, but they can hardly account for endurance of both extremes by the same individual. If such cases are found, it would seem that we must explain them by some peculiarity of the protoplasm which is not the result of adaptation. This however raises the question whether the same sort of explanation may not apply in other cases whose adaptive nature is not usually regarded as open to question.

Individuals which can endure extreme drought and extreme moisture are found among the lower plants. Some years ago the writer observed a similar case among the higher plants. The plant is a species of Tradescantia (apparently *T. fluminensis* Vell.) which grows vigorously in a saturated atmosphere, which can live submerged in water for some time, but which was nevertheless able to go without water for nearly two years. During this long period it received no moisture (except what could be absorbed from the air).

Some years ago the writer observed that pieces of this plant continued to grow when lying on a laboratory table. Curious to know how long they could live under these conditions, he placed some of them on a table before a north window without soil or other supply of moisture. Here they continued to live for nearly two years.[1] It should be noted that the air was not unusually moist. The laboratory was, on the contrary, rather dry (being situated in the third story and containing no soil or other source of moisture). The experiments were carried on at Berkeley, California. The following table shows the humidity and temperature during the greater part of the period of

[1] 1 year, 11 months, and 2 days

[The Journal for November (5: 447–506) was issued Nov. 30, 1918.]

the experiments (taken from the report of the Students' Observatory, University of California):

1907	Mean Relative Humidity	Mean Temperature
Sept.	88.3	59.0° F.
Oct.	88.7	58.0
Nov.	86.8	53.0
Dec.	89.3	49.8
1908		
Jan	90.1	48.2
Feb.	88.6	46.8
Mar.	85.0	49.9
April	84.0	54.2
May	84.0	54.9
June	85.0	56.7
July	88.0	58.6
Aug.	88.0	57.8
Sept.	86.0	58.3
Oct.	84.0	55.6
Nov.	90.0	51.1
Dec.	89.5	44.0
1909		
Jan.	92.0	49.8
Feb.	90.0	48.2
Mar.	88.0	48.1
April	85.0	54.0
May	77.0	54.4
June	84.0	58.2
July	80.0	60.2

The humidity of the air of the laboratory was less than that given in the table.

During the experiment each piece produced several new leaves. As may be observed in figure 1, these are much below the normal in size. The new leaves were formed at the expense of the older ones, which gradually died and thus furnished material which was transported to the tip of the stem and utilized in new growth.

The amount of growth is surprising when we consider how little material can be stored in the old stem and leaves. The plant shown in the photograph weighed at the start 2.1 gm. while at the end of the exposure to drought it weighed 0.22 gm. or 10.5 percent of the original weight. This loss is somewhat less than the average. In one plant

the loss amounted to 95 percent, but it remained alive and subsequently grew well when placed under normal conditions. The plant shown in the photograph was 11 cm. in length at the start. At the time the photograph was taken it measured 28 cm. Hence its gain in length during exposure to drought was 17 cm. or 150 percent.

After nearly two years of this extreme drought the plants were placed in bottles (with the lower ends of the living portions dipping in water) and transferred to a greenhouse at Cambridge, Massachusetts, where they were placed in moist soil. All of them grew vigorously, producing stems and leaves of normal size and appearance.

Some of them were placed in a saturated atmosphere in which they continued to

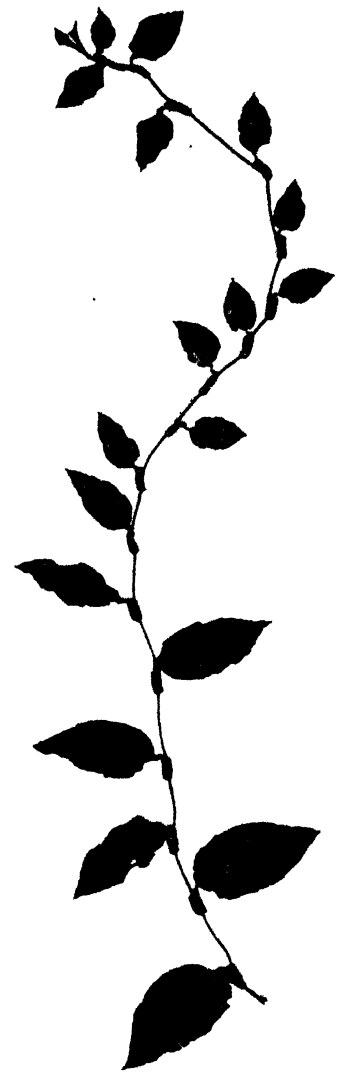


FIG. 1. Piece of Tradescantia which remained for nearly two years without soil or water. The six larger leaves at the base were present at the start. The others were formed after the plant was deprived of water and soil. During this period the plant lost 89.5 percent of its weight while at the same time it gained 150 percent in length. Subsequently it was placed in a saturated atmosphere where it grew vigorously: finally it was submerged in running water for a month, at the end of which time it was still alive.

flourish. In view of this it seemed desirable to ascertain how much moisture they could endure. For this purpose pieces eighteen inches in length were fastened at one end and anchored in a small, rapidly running brook during the month of August. The temperature of the water varied from 20° to 25°C.

Care was taken to keep all the pieces constantly submerged but not to let them sink more than an inch below the surface. The proximity to the surface and the motion of the water (which was aërated by a small waterfall directly above) ensured a fair supply of oxygen. Under these circumstances the plants grew a little (the average growth was one tenth of an inch during the month) but no new leaves were formed and all the leaves except some of the very youngest became pale and yellowish in color. The tips of some of the plants were still alive when the experiment was discontinued. Undoubtedly the plants would have done still better if it had been possible to supply more oxygen to them.

It is therefore evident that the same individual could live for nearly two years without water and afterward grow vigorously in a saturated atmosphere; in addition, it could live for a month under water and grow a little during that time.

The writer has had occasion to study other cases[2] which can not be explained by gradual adaptation. It would seem that in these cases the explanation must be sought in physical and chemical peculiarities of protoplasm which arise without reference to adaptation. It is quite possible that this kind of explanation should find more extensive application and that many cases now regarded as adaptations may prove to be fictitious.

Summary

A species of Tradescantia lived for nearly two years without soil or water: it afterward grew vigorously in a saturated atmosphere and was finally placed under water for a month, during which time it grew slightly and was alive at the end of the experiment.

The explanation of such cases must be sought in physical or chemical conditions of the protoplasm which arise without reference to direct adaptation. It would seem that the same kind of explanation may apply to many cases which are now regarded as adaptations.

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[2] *Cf.* Osterhout, W. J. V. Univ. Calif. Publ. Bot. **2**: 227. 1906. Bot. Gaz. **55**: 446. 1913.

A SIMPLE METHOD OF DEMONSTRATING THE PRODUCTION OF ALDEHYDE BY CHLOROPHYLL AND BY ANILINE DYES IN THE PRESENCE OF SUNLIGHT[1]

W. J. V. Osterhout

In 1906 it was stated by Usher and Priestly[2] that chlorophyll, extracted from leaves and spread out in thin films (under special conditions), can decompose CO_2 and produce formaldehyde in sunlight.

In the following year the writer attempted to repeat their experiments but found the method unsatisfactory; as a result a simpler method was devised which proved so successful that it has been employed by his classes ever since.

The method consists in extracting chlorophyll from fresh leaves by means of alcohol; shaking the alcohol[3] with carbon tetrachloride, drawing off the latter, sprinkling it on filter paper, and allowing it to evaporate. For this purpose the filter paper is stretched in a suitable manner (or merely hung over two parallel glass rods) and the extract sprayed over it until the paper is saturated. In the course of a few minutes, when most of the solvent has evaporated, the paper is again sprinkled with the extract. This is repeated until the paper acquires a deep green color, comparable with that of a leaf.

When the paper is dry it is placed in a bell jar, forming a lining which covers the entire surface: it is then wet with water (which incidentally helps to keep it in position by making it adhere to the glass). The bell jar is then inverted over a glass plate on which is a large petri dish containing about 5 cc. of water. The edges of the bell jar are sealed by means of vaseline. It is desirable to use a large bell jar and to cover the inside completely with filter paper. Several are prepared, some of which are placed in the light while others, kept in the dark, act as controls.

Those which are placed in the light are allowed to remain undis-

[1] An abstract appeared in Science n. ser. **42**: 68. 1915.

[2] Proc. Roy. Soc. Lond. B. **77**: 369. 1906; **78**: 318. 1906.

[3] The addition of water to the alcohol may be necessary to ensure separation.

turbed until the chlorophyll has been bleached to a pale green or a pale yellow color.[4] The bell jar is then removed and the water in the dish is tested for aldehyde by means of Schryver's test.[5] A positive result is obtained in the majority of cases.

Evidently a volatile aldehyde is produced which diffuses through the air and becomes absorbed by the water. Care is taken to place the dish so that it does not receive drippings from the filter paper. The experiment succeeds in many cases even without the use of a bell jar if the paper is simply folded into a cone and inverted over a small dish of water.

The controls kept in the dark do not give the test in any instance. It is therefore evident that if the chlorophyll contained aldehyde before exposure to sunlight the amount was too small to give a test.

It seemed desirable to ascertain whether other substances could be substituted for chlorophyll in this experiment. Accordingly a series of aniline dyes were employed. A considerable number gave positive results. The most reliable were methyl green and iodine green. Aqueous solutions were sprayed upon filter paper and allowed to dry. This was repeated until the paper was deep green in color. The paper was then exposed to sunlight in the bell jar for several days in succession until the color had practically disappeared. A positive test for aldehyde was obtained in the majority of cases, while the controls in the dark gave no test.

It therefore appeared as if the production of aldehyde was similar in the case of chlorophyll and aniline dyes, and that if the experiments of Usher and Priestly had any bearing on photosynthesis the experiments on aniline dyes were of considerable interest.

At that time no criticisms of Usher and Priestly's work had appeared, and the writer undertook experiments to test the validity of their conclusions regarding photosynthesis. These experiments showed

[4] This may require several days.

[5] The test is made as follows: Add to 10 cc. of the solution to be tested:

2 cc. of 1 percent phenylhydrazine hydrochloride (freshly prepared and filtered).

1 cc. 5 percent potassium ferricyanide (freshly prepared).

5 cc. concentrated HCl.

On adding a drop of amyl alcohol or chloroform and shaking vigorously, the pinkish color will become concentrated in the chloroform.

The test is given by a variety of aldehydes. *Cf.* Schryver, S. B. Proc. Roy. Soc. Lond. B. 82: 226. 1910.

that it makes practically no difference in the result whether CO_2 is excluded altogether from the bell jar or whether the concentration is increased to 10 percent but it was found that the presence of oxygen is necessary. The writer therefore came to the conclusion that the aldehyde is not produced by the decomposition of CO_2 but rather by the decomposition of chlorophyll.

Subsequently a number of criticisms of the work of Usher and Priestley have appeared, to which these authors have replied.[6] Recently several authors[7] have stated that the aldehyde produced in Usher and Priestley's experiments is the result of oxidative decomposition of chlorophyll. Wager and Ewart believe that decomposition of chlorophyll, and the resulting formation of aldehyde, is a regular step in photosynthesis. There is some evidence in favor of this view,[8] but at present it cannot be said that there is any convincing proof of its correctness.

Summary

1. A method is described by which aldehyde is obtained from chlorophyll in sunlight; the yield is relatively large and it is free from contamination by non-volatile substances.

2. A similar production of aldehyde is observed when certain aniline dyes are substituted for chlorophyll.

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[6] *Cf.* Usher, F. L., and Priestley, J. H. Proc. Roy. Soc. Lond. B. **84**: 111. 1911.

[7] *Cf.* Wager, H. Proc. Roy. Soc. Lond. B. **87**: 386, 596. 1914; Warner, C. H. Ibid. **87**: 378. 1914; Ewart, A. J. Ibid. **89**: 1. 1915; Jorgensen, I., and Kidd, F. Ibid. **89**: 342. 1916. For the decomposition of other substances in light, with production of aldehyde, see Neuberg, C. Biochem. Zeit. **13**: 305. 1908; Spoehr, H. A. Biochem. Zeit. **57**: 95. 1913.

[8] That chlorophyll or some compound of it may decompose during photosynthesis is indicated by a recent study of the dynamics of the process. See Osterhout, W. J. V., and Haas, A. R. C. Proc. Nat. Acad. Sci. 4: 85. 1918. Journal of General Physiology 1[1]. 1918.

OREOMYRRHIS BORNEENSIS MERR. SP. NOV., AN INTERESTING ADDITION TO OUR KNOWLEDGE OF THE MALAYAN FLORA

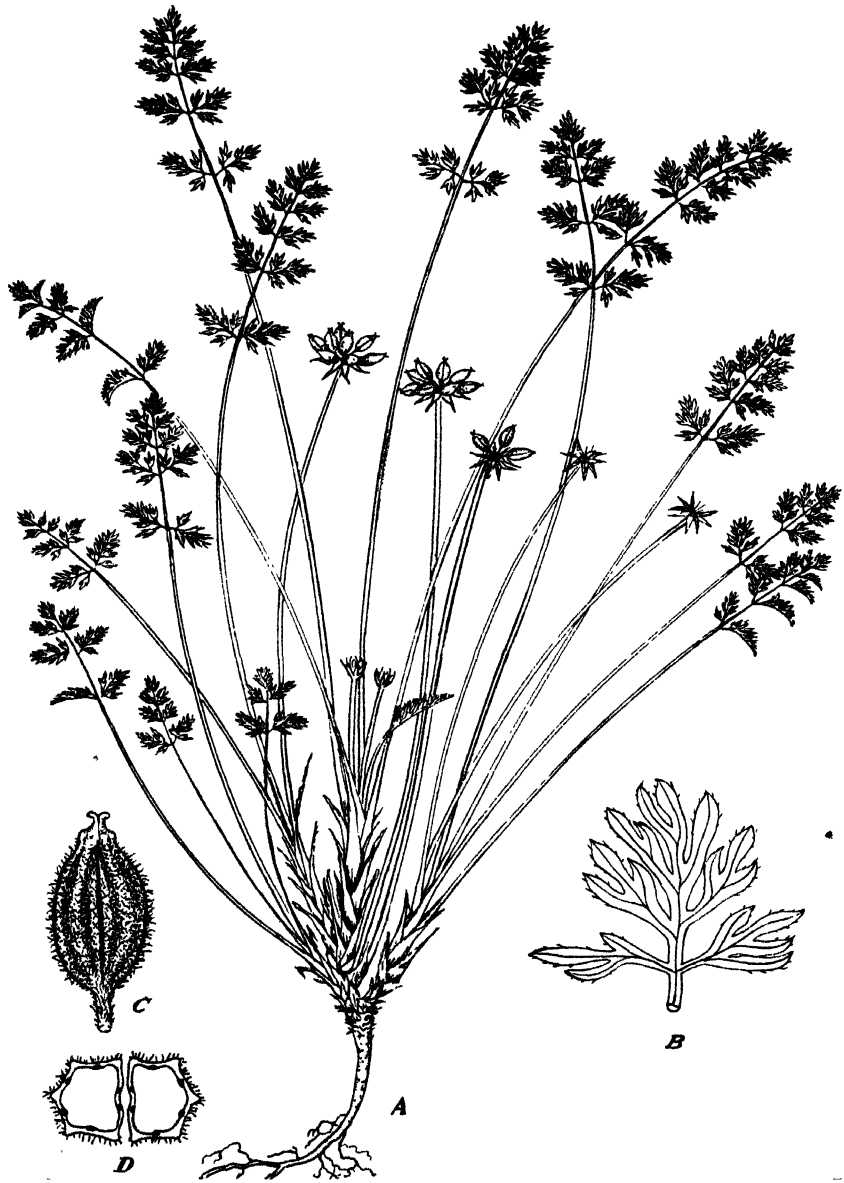
E. D. MERRILL

The discovery of a representative of the genus Oreomyrrhis Endlicher on Mount Kinabalu, the highest mountain in the Malayan region outside of New Guinea, adds another species to the rather remarkable list of plants now known from that mountain which must be considered as Australian types. It is, moreover, the first representative of this small genus to be found in the Malayan region, or for that matter in the Old World north of Australia. Other Kinabalu species indicating unmistakable Australian or Australian-New Zealand alliances include *Blechnum fraseri* Luerss., *Ranunculus lowii* Stapf, *Drimys piperita* Hook. f., *Didiscus saniculaefolius* (Stapf) Merr., *Coprosma crassicaulis* Stapf, *C. hookeri* Stapf, *Nertera depressa* Banks, *Lagenophora gibbsiae* Merr., *Gaultheria borneensis* Stapf, *Euphrasia borneensis* Stapf, *Drapetes ericoides* Hook. f., *Patersonia lowii* Stapf, *P. borneensis* Stapf, *Centrolepis kanibaluensis* Gibbs, *Scirpus inundatus* Spreng., *Schoenus kinabaluensis* Stapf, and *S. melancostachyus* R. Br. Representatives of all the characteristically Australian genera of the above list, with the exception of Coprosma and Drapetes, and of Oreomyrrhis, have been found in the Philippines, but no representatives of the genera Schoenus, Drimys, Didiscus, Drapetes, Patersonia, Centrolepis, or Oreomyrrhis have been found in western Malaya, although some have been found in Celebes and in New Guinea, a distribution wholly to be expected of all of them.

Oreomyrrhis borneensis sp. nov.

Herba caespitosa, glabra vel subglabra, usque ad 15 cm. alta; foliis longe petiolatis, in ambitu oblongis, usque ad 4 cm. longis et 1.5 cm. latis, bi-tripinnatim dissectis, segmentis numerosis, parvis, oblongis, acuminatis, 1 ad 2 mm. longis, 0.3 ad 0.5 mm. latis; pedunculis erectis, quam petiolis brevioribus, sursum parce pubescentibus, tenuibus; floribus paucis (circiter 10), breviter pedicellatis; fructibus oblongis, acuminatis, 3 ad 4 mm. longis, distincte cinereo-hirsutus, carpellis distanter 5-costatis.

A tufted perennial herb 8 to 15 cm. high, simple or with few basal



MERRILL: OREOMYRRHIS BORNEENSIS SP. NOV.

branches which are short and densely crowded, and covered with the persistent dry sheathing basal parts of the petioles, the plant glabrous or subglabrous. Leaves long-petioled, the petioles glabrous, slender, up to 10 cm. in length, the margins of the basal sheathing parts somewhat ciliate; lamina in outline oblong, 1.5 to 4 cm. long, 0.7 to 1.5 cm. wide, bi-tripinnately dissected, the segments small, rather rigid, oblong, numerous, 1 to 2 mm. long, 0.3 to 0.5 mm. wide, their margins sometimes very obscurely ciliate, apices acute to apiculate-acuminate. Peduncles shorter then the petioles, reaching a maximum length of 7 cm. in fruit, in anthesis not more than 3 cm. long and then much more pubescent than when in fruit, the hairs short, cinereous, never reflexed. Involucral bracts about 10, oblong-lanceolate, acuminate, somewhat pubescent, about 3 mm. long, 0.5 to 0.8 mm. wide. Flowers about 10, pinkish, short-pedicelled, much shorter than the bracts. Petals ovate to broadly elliptic-ovate, about 0.8 mm. long, the basal margins slightly ciliate. Fruits oblong, 3 to 4 mm. long, narrowed upward, acuminate, distinctly cinereous-hirsute with short hairs, the carpels distantly 5-ribbed, the pedicels very slightly or not at all elongated in fruit. Vittae as in other species of the genus, one under each furrow and two toward the commissure, the commissural side of the albumen merely slightly concave, not furrowed.

British North Borneo, Mount Kinabalu, Low's Peak, *Mrs. Clemens 10622* (type), *Topping 1687*, November 13, 1915, noted in two crevices near the summit, associated with Carex, altitude about 4,000 meters.

I have before me several New Zealand and Australian specimens of Oreomyrrhis representing as many different forms or varieties of *Oreomyrrhis andicola* Endl. as there are specimens. I cannot consider the Kinabalu specimen to be specifically identical with any of these forms, the several species described from Australia and from New Zealand having, by common consent, all been reduced to the South American *O. andicola* Endl., thus giving us but a single species of the small genus in the Old World. The species above described distinctly approaches a New Zealand form from Awatere, distributed by H. H. Travers as *Oreomyrrhis andicola* Endl. forma *tenuifolia*. It differs radically from this form, however, in its very long petioles; in its peduncles being shorter than the petioles, the New Zealand form having the peduncles much longer than the leaves; in its very short pedicels and in its cinereous-hirsute, not glabrous fruits.

EXPLANATION OF PLATE XXXVI

Oreomyrrhis borneensis Merr. sp. nov. *A*, an entire plant, natural size; *B*, a pinna, × 5; *C*, a fruit, × 7; *D*, cross section of a fruit, × 12.5.

ROOT ABSORPTION FROM SOLUTIONS AT MINIMUM CONCENTRATIONS

R. B. HARVEY AND R. H. TRUE

In former papers by R. H. True and H. H. Bartlett (1, 2) there was found to be a point in the absorption curve for the lupine grown in various salt solutions, at which the absorption and excretion of electrolytes were in equilibrium. For the lupine this value was found to be about 16 x 10^{-6} normal expressed as NaCl. The conditions which exist at this point have been further studied in the squash, peanut, and soy bean. The values have been found to differ slightly for the various plants used.

The absorption minimum for the same plant seems to have nearly the same value independent of the volume of the solution, the concentration of the salt, or the kind of nutrient salt used. The quantity of salt solution offered must, of course, be within the requirements of the plant during its growth period, so that the minimum may be reached, and the concentration must be below the toxic limit. The constancy of the equilibrium concentration when once attained is shown by the graph for sweet corn in calcium nitrate (fig. 1). The value of 12–15n x 10^{-6} $Ca(NO_3)_2$ was held for 24 days by five cultures in this series. In cultures of this plant grown in KNO_3 and $Mg(NO_3)_2$, practically the same value was obtained. The graph for the absorption of the squash in $Ca(NO_3)_2$ (fig. 2) gives the average values for twelve cultures at each concentration indicated. The squash seems unable to maintain a concentration as low as corn, giving a value of 35–40n x 10^{-6} $Ca(NO_3)_2$ as its absorption minimum. The peanut is able to maintain a concentration of 50n x 10^{-6}; the soy bean one of about 20n x 10^{-6} expressed as $Ca(NO_3)_2$. Sweet corn gave a more nearly constant value than the other plants tested.

The specific minimum concentration for absorption is controlled by at least two classes of factors. One external factor, the concentration of CO_2 in the air and in the solution saturated with it at the given temperature, like other similar purely external factors, must be the same for different plants growing under the same conditions.

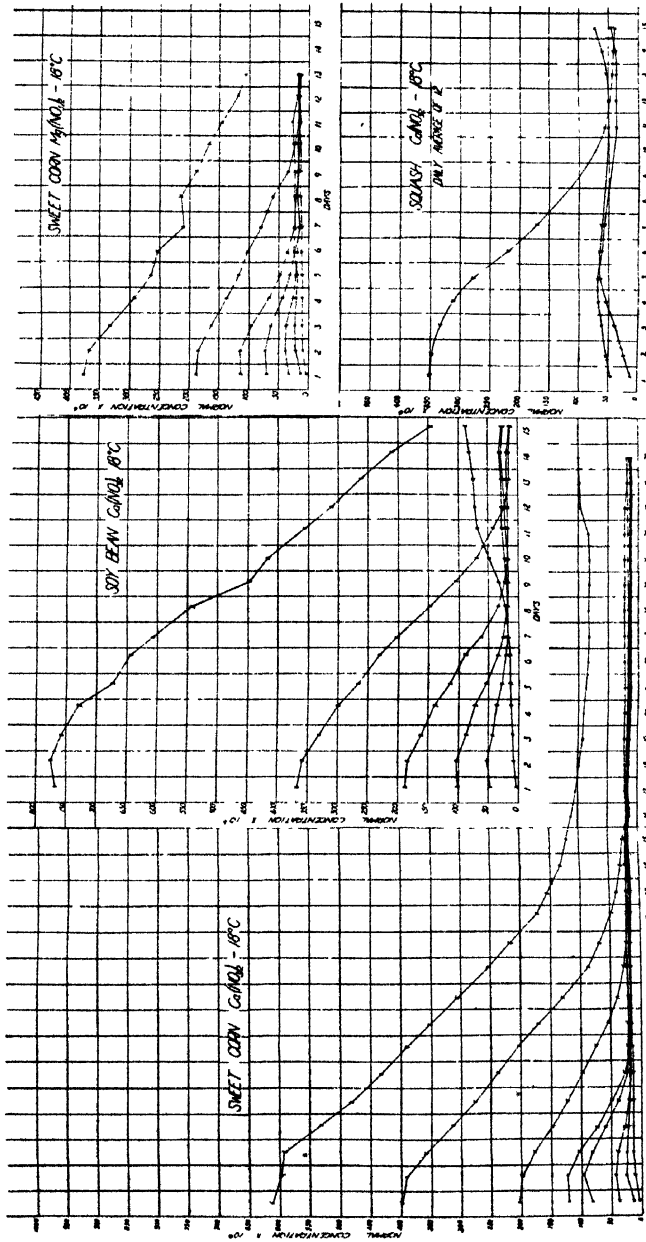


FIG. 1. Graph showing rates of root absorption from solutions of $Ca(NO_3)_2$ and $Mg(NO_3)_2$.

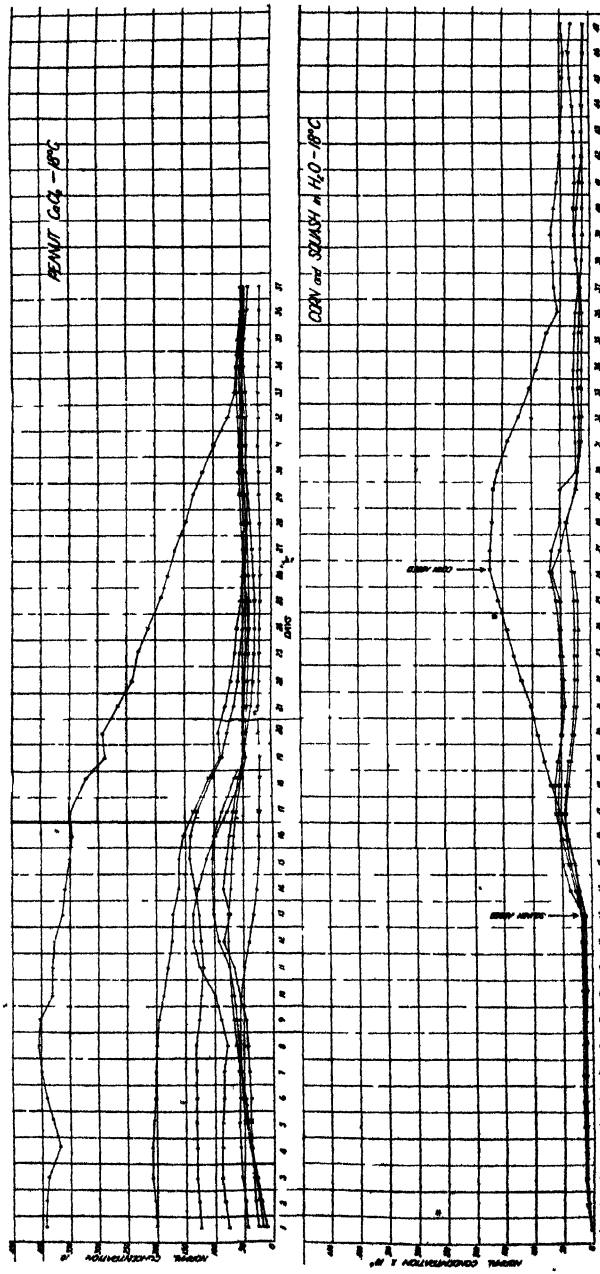


FIG. 2. Graph showing rates of root absorption from water and from solutions of $CaCl_2$.

Another class of factors depends upon the plant for their values. Such would be the chemical character of the cell wall and the deeper lying cell membranes and other structures. These membranes contain dissociating substances, such as calcium pectate and many other substances (3). The value of the minimum concentration for absorption must be determined by the balance of ion interchange between the membrane surface and the solution. It would seem likely that when the syntheses within the cell which tend actively to withdraw ions toward the interior is balanced by the tendency toward dissociation and loss of ions at the bounding membrane, the condition here seen would be realized.

Since both of the internal factors here mentioned depend on structure and function supposed to be specific, it is hardly surprising that this minimum concentration should also be specific. To test this point corn was grown in conductivity water for 10 days after equilibrium had been established. For six cultures of five plants each the variation during this period was $10\text{–}18n \times 10^{-6}$ expressed as KCl. At the end of that time the solution in one beaker was boiled to remove CO_2, made up to its original weight with recently distilled conductivity water, stoppered to prevent the absorption of CO_2, and read at 18° C. The total concentration before boiling was $11 \times 10^{-6}n$ as KCl. The CO_2 concentration was $6 \times 10^{-6}n$ as KCl, leaving a residual concentration of $5 \times 10^{-6}n$ of non-volatile electrolytes which the corn is unable to absorb. Squashes were then placed in the set-up. That the solution was not in equilibrium with the squash was shown by the rise in concentration, much as is seen in distilled water. After a period of leach and subsequent absorption, equilibrium was established at a concentration higher than the value for corn. Infection occurred in one culture, a fact which became apparent in the continual leach of electrolytes. The beaker having the lowest value was tested as before. The concentration before boiling was 28.7, after boiling $22.3n \times 10^{-6}$ KCl, leaving a residual concentration of $6.4n \times 10^{-6}$ expressed as KCl, which is due to volatile electrolytes. The close agreement with the former value for CO_2 is apparent as well as the higher average value for the equilibrium concentration maintained by the squash. The squash was again replaced by corn and the concentration sank to its former value for corn in all cultures except the one which became infected.

In a recent article Stiles (4) says: "It is quite conceivable that

below a certain point, concentrations of the nutrient solution might be so low that the root could not absorb enough salts from the solution simply because of the dilution of the latter." This surmise seems entirely correct in view of the data here presented.

To maintain so low a value for the ion content of the solution about it for three weeks, as is here seen in sweet corn, either the cell membrane must possess almost perfect directional semipermeability and be able to hold the electrolytes during life, or the ions present must be chemically combined into compounds which are but little dissociated. The variation in the deviation from the value for CO_2 saturation indicates specific differences in the rate of ion production. Since upon the death of the plant leaching occurs, the assumed directional permeability is to be ascribed to some living membrane of the plant, or the leach is due to the breaking down of ion compounds of the cell substances upon the death of the protoplasm.

SUMMARY

The equilibrium concentration of electrolytes established by the squash, peanut, soy bean, and sweet corn grown in water culture was found to be specific for each plant.

The equilibrium concentration value is independent of the kind of nutrient salt used, the concentration of the electrolyte, or the volume of the solution; provided, that the concentration is below the toxic limit for the plant, and that the quantity of the salt is within the requirement of the plant during the period of growth.

At the point of equilibrium between the plant and the solution, the electrolyte content of the solution is determined, first, by certain factors which are constant for different plants under the same conditions, such as the CO_2 equilibrium with the air; and, second, it is determined by the rate of cleavage of ion-producing compounds of the cell and the reabsorption of the ions produced.

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UREDINALES OF GUATEMALA BASED ON COLLECTIONS BY E. W. D. HOLWAY

IV. PUCCINIA ON CARDUACEAE, FORM-GENERA, AND INDEX

J. C. ARTHUR

The preceding parts of this account of Guatemalan rusts were published in this journal (June, 1918, pp. 325–336; October, 1918, pp. 420–446; November 1918, pp. 462–489). With the present concluding part an index both to rusts and hosts is provided to facilitate ready reference.

The composites of the tropics are both numerous and diversified. In many of the genera are intergrading forms. The composite rusts are also numerous and in many cases most difficult to delimit, often showing variations comparable with those of the hosts. The material of this part has been reviewed and, when required, critically studied by Professor H. S. Jackson, who has drawn up the diagnoses for the eight new species. The composite collections of Professor Holway from Costa Rica were studied at the same time and the results published in a paper by the writer on the Costa Rican rusts in Mycologia (10: 111–154. 1918).

The species here remaining in form-genera are not so numerous as is usually the case with tropical rusts. All but two or three of them evidently belong to the Aecidiaceae, and the other stages can be expected to turn up before very long.

For this fine showing of Guatemalan rusts chief credit is due to Professor E. W. D. Holway, who has given abundantly of his time and private means to carry out the explorations, and who has also co-operated in the study of the material. Grateful acknowledgment is also to be made to the officers and mycologists of the Purdue University Agricultural Experiment Station who have provided facilities for making the microscopic examinations and have assisted in the studies.

178. PUCCINIA INSULANA (Arth.) Jacks. & Holw. (on Carduaceae).

Vernonia sp., Retalhuleu, Feb. 26, 1916, O, II_1, II_2, III, *537*.

The species has been known heretofore from the West Indian islands

on *V. albicaulis* and *V. longifolia* under the name *Argomyces insulanus* Arth., and is now reported for the first time from the continent.

179. PUCCINIA ERRATICA Jacks. & Holw. (on Carduaceae).

Vernonia Schiedeana Less., Guatemala City, Feb. 15, 1916, O, I, II, III, *494*; same, Feb. 8, 1917, O, I, ii, III, *841*; Chinautla, Dept. Guatemala, Feb. 12, 1916, O, I, ii, iii, *480*; Moran, Dept. Amatitlan, Dec. 22, 1916, I, II, iii, *621*.

The aecia of this species were described as *Dietelia Vernoniae* Arth. (Bot. Gaz. **40**: 198. 1905), afterward transferred to the genus Endophyllum, as *E. Vernoniae* Arth. (N. Am. Flora **7**: 126. 1907), from a Mexican specimen thought to be on *Vernonia Deppeana*, but which on careful comparison with the Guatemalan material seems to be *V. Schiedeana*. Re-examination of the type of *E. Vernoniae*, furthermore, reveals a few urediniospores and teliospores which agree perfectly with the present material. Further confirmation of the long-cycle character of the rust was found on a specimen of *V. Schiedeana* collected by C. G. Pringle at Cordoba, Mexico, no. *6080*, in the phanerogamic collection of the New York Botanical Garden, which gave all the spore forms, although the teliospores are a little shorter and broader than usual, doubtless due to the more mature character of the host.

The species is Eriosporangium-like, and the absence of a peridium in the aecia and the deciduous sculpturing of the aeciospores are in accordance with the early ideas regarding that genus, as well as the thin-walled spores, which germinate upon maturity. A new name has been chosen for this species, owing to the priority of the very dissimilar *Puccinia Vernoniae* Schw., founded in 1832.

180. PUCCINIA NOTHA Jacks. & Holw. (on Carduaceae).

Vernonia leiocarpa DC., San Rafael, Dept. Guatemala, Jan. 7, 1915, III, *21*; Solola, 7000 feet alt., Jan. 28, 1915, I, II, III, *148*; Guatemala City, Feb. 15, 1916, III, *495*, intermixed with another species; March 17, 1916, III, *585a*, being intermixed with, and separated from the type collection of *P. rata*; Volcan de Agua, Dept. Sacatépequez, March 4, 1916, III, *550*; Quezaltenango, Jan. 16, 1917, I, II, III, *732*; Huehuetenango, Jan. 21, 1917, I, II, III, *759*.

Vernonia Shannoni Coult. (?), Quezaltenango, Jan. 31, 1917, III, *814*.

In the collection from Quezaltenango, no. *732*, the telia are about equally abundant on both surfaces of the leaf and the teliospores have shorter pedicels than in the other collections. The species is nearest to *P. idonea*.

181. PUCCINIA RATA Jacks. & Holw. (on Carduaceae).

Vernonia leiocarpa DC., Guatemala City, Feb. 13, 1916, ii, III, *490*; same, Feb. 15, 1916, II, III, *495a*, intermixed with *P. notha*; same, March 17, 1916, II, III, *585*, intermixed with *P. notha*; Mendez, Dept. Guatemala, Feb. 13, 1917, III, *860*.

A species readily separable from others at present known on Vernonia by the paraphysate uredinial sori and tuberculate teliospores. On some leaves it is accompanied by *P. notha*, from which it may be distinguished by the position on the under surface of the leaf, the paraphysate sori, the dark-colored urediniospores and tuberculate teliospores. No aecia have yet been found. The species is known only from Guatemala.

182. PUCCINIA IDONEA Jacks. & Holw. (on Carduaceae).

Vernonia trifloscculosa H.B.K., Chinautla, Dept. Guatemala, Feb. 12, 1916, ii, III, *481*; Escuintla, Feb. 17, 1916, II, iii, *498*; same, II, III, *499*; Panajachel, Dept. Solola, Jan. 3, 1917, ii, III, *670*.

The type selected for this species was collected in Costa Rica, on *Vernonia trifloscculosa* H.B.K., at San José, Jan. 18, 1916, by E. W. D. Holway *445*. No pycnia were found in either the Costa Rican or Guatemalan collections, and the nature of the complete life cycle remains uncertain. The species is similar to *P. notha*, but has smaller and narrower urediniospores, with hemispherical and closely set projections on the teliospores.

183. PUCCINIA PRAEALTA Jacks. & Holw. (on Carduaceae).

Vernonia trifloscculosa H.B.K., Mazatenango, Dept. Suchitepequez, Feb. 21, 1916, II, III, *510*.

A very distinct species for which the aecia are not known, separable from all others on the genus Vernonia by the very deep-seated and strictly epiphyllous sori. The gross appearance is that of a microform. It occurs also in Costa Rica.

184. PUCCINIA INAEQUATA Jacks. & Holw. (on Carduaceae).

Vernonia patens H.B.K., Sanarate, Dept. Guatemala, Feb. 10, 1916, II_2, III, *470*; Escuintla, Feb. 17, 1916, O, II_1, II_2, III, *502*; Mazatenango, Feb. 22, 1916, II_2, *513*; Retalhuleu, Feb. 26, 1916, O, II_1, *534*; Agua Caliente, Dept. Guatemala, Feb. 10, 1917, II_2, III, *851*.

The same rust was also found on *V. patens* from Guatemala, in the phanerogamic herbarium at the New York Botanical Garden, showing uredinia and telia, having been collected at Santa Rosa, February, 1893, by Heyde & Lux *4524*. It is a long-cycle species with all spore forms, not known outside of Guatemala.

185. PUCCINIA DISCRETA Jacks. & Holw. (on Carduaceae).

Vernonia Deppeana Less., San Felipe, Dept. Retalhuleu, Jan. 14, 1917, O, III, *721*; Colomba, Dept. Quezaltenango, Feb. 2, 1917, *818*.

Type is on *Vernonia Deppeana* Less, San José, Costa Rica, collected by E. W. D. Holway, Dec. 15, 1915, no. *260*. The rust has a characteristic gross appearance and usually occurs on the leaves of the terminal shoots of young plants. It is a short-cycle micro-form with pycnia.

186. PUCCINIA PAUPERCULA Arth. (on Carduaceae).

Elephantopus spicatus Juss., Mazatenango, Dept. Suchitepequez, Feb. 21, 1916, *510A*; same, Feb. 25, 1916, *530*.

A short-cycle species known heretofore only from Mexico and Costa Rica.

187. PUCCINIA CONOCLINII Seym. (on Carduaceae).

Ageratum conyzoides L., San Felipe, Dept. Retalhuleu, Jan. 12, 1917, II, *697*.

Ageratum corymbosum latifolium (DC.) Robinson, Chinautla, Dept. Guatemala, Feb. 12, 1916, II, iii, *482*; Moran, Dept, Amatitlan, Dec. 22, 1916, II, III, *623*.

Ageratum rugosum Coult., Antigua, Dept. Sacatépequez, Jan. 12, 1915, II, III, *74*.

Eupatorium collinum DC., Guatemala City, Dec. 23, 1916, II, III. *627*; Huehuetenango, Jan. 21, 1917, ii, III, *757*.

Eupatorium glandulosum H.B.K. (?), Quezaltenango, Jan. 31, 1917, ii, III, *810*.

Eupatorium Neaeanum DC., Solola, Jan. 27, 1915, II, III, *131*.

Eupatorium pycnocephaloides Robinson, Volcan de Agua, Dept. Sacatépequez, Jan. 13, 1915, II, III, *83*; same, March 7, 1916, II, III, *564*; Solola, 7000 feet alt., Jan. 28, 1915, II, III, *144*; Quezaltenango, Jan. 18, 1917, II, III, *750*, Huehuetenango, Jan. 23, 1917, II, III, *774*.

Eupatorium pycnocephaloides glandulipes Robinson, Totonicapam, Jan. 24, 1915, ii, III, *106*.

Eupatorium pycnocephalum Less., Solola, Jan. 29, 1915, II, III, *153*; Antigua, Dept. Sacatépequez, March 2, 1916, II, III, *549*: San Felipe, Dept. Retalhuleu, Jan. 13, 1917, II, 713.

Eupatorium sp., Guatemala City, Dec. 23, 1916, II, III, *629*; San Felipe, Dept. Retalhuleu, Jan. 14, 1917, II, *717*; Aguas Amargas, Dept. Quezaltenango, Jan. 30, 1917, II, III, *801*.

This long-cycle rust is imperfectly known. It is presumable that the species possesses aecia as well as pycnia, although neither have yet been seen. This is the more likely as no rust on Eupatorium or its close allies has yet been found with pycnia associated with the uredinia. For a time it was supposed that the aecia on Eupatorium from Mexico and Central America, having apically thickened walls, belonged with this species, and since 1906 the combination has often been called "*Puccinia rosea*." By the observations of Prof. Holway, coupled with data regarding distribution, it now seems reasonably certain that the aecia in question are heteroecious, and belong with a grass species (see no. 117).

The rust was collected by Kellerman on *Ageratum conyzoides*, at Mazatenango, Feb. 28, 1905, II, *4346*, *5373*, and at San Felipe, Feb. 4, 1906, II, *5446* (Kellerm. Fungi Sel. Guat. *7*); and on *Eupatorium pycnocephalum*, at Guatemala City, Feb. 1, 1905, II, iii, *5312*. The three collections were reported by Kern in Journ. Myc. *l.c.*

188. **Puccinia Hodgsoniana** Kern sp. nov. (on Carduaceae).

Eupatorium Schultzii Schnitt., forma *erythranthodium* Robinson, Agua Caliente, Dept. Guatemala, Feb. 10, 1917, II, III, *853*.

Eupatorium Schultzii ophryolepis Robinson, San Lucas Toliman, 7000 feet alt., Dept. Solola, Feb. 3, 1915, II, III, *187*; Quezaltenango, Jan. 18, 1917, II, III, *744*; Agua Amargas, Dept. Quezaltenango, Jan. 30, 1917, II, III, *804*.

Eupatorium Schultzii velutipes Robinson, San Lucas Toliman, 5100 feet alt., Dept. Solola, Feb. 2, 1915, II, iii, *170*; Guatemala City, March 17, 1916, II, *587*.

Uredinia hypophyllous, scattered, round, 0.3–0.5 mm. across, early naked, pulverulent, chestnut-brown, ruptured epidermis conspicuous; urediniospores globoid to obovoid, 19–26 by 24–30 μ; wall chestnut-brown, 1.5–2.5 μ thick, moderately and finely echinulate, the pores 2, near the hilum, or rarely 3, all near the hilum or one of the three near the apex.

Telia chiefly hypophyllous, scattered, small, round, 0.5–0.8 mm. across, early naked, pulverulent, blackish-brown, ruptured epidermis inconspicuous; teliospores oblong or ellipsoid, 24–29 by 40–45 μ, rounded or obtuse above, rounded below, scarcely constricted at septum; wall chestnut-brown, 3–4 μ thick, lighter colored and thicker at apex, 5–9 μ, equally thickened over pore of lower cell, closely and prominently verrucose; pedicel colorless, once to twice length of spore, sometimes attached obliquely.

The type of the species is a collection by Kellerman *6087*, made Feb. 6, 1907, on Volcan Acatenango, 6000 feet alt., Dept. Sacatépequez, on *Eupatorium phocnicolepis guatemalensis* Robins., the host being determined by J. M. Greenman, and the name of the fungus attached by Dr. F. D. Kern.

189. **Puccinia solidipes** Jacks. & Holw. sp. nov. (on Carduaceae).

Eupatorium tubiflorum Benth., San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 7, 1915, ii, III, *18*; Volcan de Agua, Dept. Sacatépequez, March 4, 1916, ii, III, *557* (type); Zunil, Dept. Quezaltenango, Jan. 28, 1917, ii, III, *793*.

Uredinia hypophyllous, scattered, or somewhat gregarious, round, small, 0.1–0.3 mm. across, early naked, pulverulent, cinnamon-brown, ruptured epidermis barely noticeable; urediniospores globoid or obovoid, 23–29 by 26–32 μ; wall dark cinnamon-brown, thin, 1–1.5 μ, closely and finely echinulate, the pores 2, sometimes 3, approximately equatorial.

Telia amphigenous, scattered, 0.5–1 mm. across, early naked, somewhat pulverulent, blackish-brown, ruptured epidermis barely noticeable; teliospores broadly ellipsoid, 30–35 by 38–45 μ, rounded at both ends, slightly constricted at septum; wall chestnut-brown, rather thick, 2.5–4 μ, slightly thicker above by a lighter umbo, 5 6 μ; pedicel colorless, persistent, the wall thickened often nearly obliterating the lumen, the surface granulose at base, twice to thrice length of spore, 6–7 μ in diameter.

This species differs conspicuously from *P. inanipes* Diet. & Holw., with which it has been confused, by having urediniospores of the usual globoid or obovoid form, while in *P. inanipes* they are strongly flattened above and below, forming an oblate spheroid, and also by the

solid, or nearly solid, pedicels of the teliospores, caused by the greatly thickened walls, as well as by minor characters.

The same rust on the same host was collected by Kellerman at Volcan de Atitlan, Dept. Solola, Feb. 16, 1906, ii, III, *5314*, and was reported by Kern in Mycologia *l.c.*, under the name *P. inanipes* It was collected by Prof. Holway on the same host at Patzcuara, Mexico, Oct. 17, 1898, *3007*, Oct. 19, 1898, *3232*, and Oct. 10, 1899, *3600*. These three collections bear uredinia but no telia. These specimens were collected for the *Aecidium roseum* Diet. & Holw., which they bear in abundance, while the uredinia are less conspicuous. The aecia were also taken at the same locality and time on other species of Eupatorium, and are believed to be heteroecious (see no. 117). The characters for the uredinial sorus, given above, are drawn from the Mexican material, the other characters from the Guatemalan material.

190. **Puccinia basiporula** Jacks. & Holw. sp. nov. (on Carduaceae).

Eupatorium Mairetianum DC., Quezaltenango, Jan. 16, 1917, II, III, *733*; same, Jan. 31, 1917, II, III, *808*; same, Feb. 4, 1917, II, *837*.

Eupatorium Mairetianum adenopodum Robinson, Cerro Quemado, Dept. Quezaltenango, Jan. 21, 1915, ii, III, *98* (type).

Uredinia hypophyllous, scattered, round, small, 0.2–0.3 mm. across, early naked, pulverulent, cinnamon-brown, ruptured epidermis noticeable; urediniospores globoid, sometimes flattened at hilum, 21–24 μ in diameter; wall cinnamon-brown, thin, 1–1.5 μ, closely and finely echinulate, pores 2, near the hilum, often indistinct.

Telia chiefly hypophyllous, scattered, round, small, 0.3–0.5 mm. across, early naked, somewhat pulverulent, blackish-brown, ruptured epidermis inconspicuous; teliospores ellipsoid, 23–26 by 32–35 μ, rounded at both ends, slightly constricted at septum; wall chestnut-brown, 1.5–2.5 μ, thickened at apex and over pore of lower cell to 5 μ, closely and distinctly verrucose; pedicel colorless, firm, 7 μ thick, once and a half to twice length of spore, often attached obliquely, the wall thin.

The species was collected by Kellerman, on *E. rafaelense* Coult., at Volcan de Cerro Quemado, Feb. 8, 1906, III, *5449*, and reported by Kern in Journ. Myc. *l.c.*, under the name of *P. Conoclinii*, and likewise issued in Kellerm. Fungi Sel. Guat. *14*.

191. PUCCINIA TOLIMENSIS Mayor (on Carduaceae).

Eupatorium pansamalense Robinson, Agua Amargas, Dept. Quezaltenango, Jan. 30, 1917, *802*.

Eupatorium sp., San Rafael, Dept. Guatemala, Jan. 7, 1915, *22*; Aguas Amargas, Dept. Quezaltenango, Jan. 30, 1917, *806*.

A short-cycle South American species not before reported from North America.

192. PUCCINIA BACCHARIDIS Diet. & Holw. (on Carduaceae).

Baccharis glutinosa Pers., Chinaulta, Dept. Guatemala, Jan. 17, 1915, O, I, ii, *91*; Panajachel, Dept. Solola, Jan. 30, 1915, o, i, II, III, *158*.

A long-cycle rust, with all spore forms, placed in the North American Flora (7: 213) under the genus Eriosporangium, as *E. punctato-striatum* (Diet. & Neg.) Arth.

193. PUCCINIA EXORNATA Arth. (on Carduaceae).

Baccharis rhexioides H.B.K., San Lucas Toliman, 5100 feet alt., Dept. Solola, Feb. 2, 1915, O, I, ii, III, *174*; Guatemala City, Feb. 8, 1916, o, i, II, III, *462*; Mendez, Dept. Guatemala, Feb. 13, 1917, O, I, II, III, *863*.

The aeciospores and urediniospores of this collection are somewhat narrower, and the former thinner-walled, than in the type material. The type collection was made by Kellerman at Guatemala City, on *B. thesioides* H.B.K., Feb. 2, 1905, O, I, II, III, *5368*. The present collections are the first made since the original one was taken.

194. PUCCINIA ANCIZARI Mayor (on Carduaceae).

Baccharis lancifolia Less., Cerro Quemado, Dept. Quezaltenango, Jan. 21, 1915, O, I, III, *103*; Tecpan, Dept. Chinaltenango, Jan. 1, 1917, o, i, III, *660*.

This long-cycle species is without uredinia. It was described by Mayor in 1913 from material collected in Colombia on *Baccharis nitida*, and is now first reported from North America.

195. PUCCINIA BACCHARIDIS-MULTIFLORAE Diet. & Holw. (on Carduaceae).

Baccharis serraefolia DC., Solola, Jan. 25, 1915, II, *115*; same, 6000 feet alt., Jan. 27, 1915, II, *123*; Huehuetenango, Jan. 23, 1917, II, *770*.

Baccharis sp., Guatemala City, Jan. 9, 1917, II, *687*; Quezaltenango, Jan. 16, 1917, II, *731*.

A long-cycle species possessing pycnia, uredinia, and telia, heretofore reported only from Mexico, and on other species of hosts.

196. PUCCINIA OAXACANA Diet. & Holw. (on Carduaceae).

Conyza asperifolia (Benth.) Benth. & Hook. (*Baccharis hirtella* DC.), San Rafael, Dept. Guatemala, Jan. 7, 1915, I, *32*; same, Jan. 9, 1915, ii, III, *46*; Colomba, Dept. Quezaltenango, 3 Feb., 1917, III, *826*.

The aecia in no. *32* of this long-cycle rust are not in small groups on the leaves, as usually seen, but on the axillary buds, causing them to become greatly hypertrophied, making an etiolated mass 1–2 cm. long, and thickly covered with the aecia. The rust is often listed as *Eriosporangium oaxacanum* (Diet. & Holw.) Arth.

197. PUCCINIA NOCCAE Arth. (on Carduaceae).

Lagascea suaveolens H.B.K., Guatemala City, Jan. 3, 1915, II, *12a*; same, Feb. 8, 1916 II, *463*; Solola, 6000 feet alt., Jan. 30, 1915, II, iii, *155*.

A long-cycle rust, whose primary form is unknown. Heretofore it has been recorded only from Mexico.

198. PUCCINIA CALEAE Arth. (on Carduaceae).

Calea Zacatechichi Schlecht., Antigua, Dept. Sacatépequez, Dec. 28, 1916, III, *643*.

Calea Zacatechichi macrophylla Robins. & Greenm., Guatemala City, Jan 1, 1915, ii, III, *7*; Chinaulta, Dept. Guatemala, Jan. 17, 1915, III, *89*; Solola, Jan. 27, 1915, III, *132*.

Calea sp., Panajachel, Dept. Solola, Jan. 3, 1917, III, *675*.

A long-cycle rust, with all spore forms, known heretofore only from Mexico.

199. **Puccinia ordinata** Jackson & Holw. sp. nov. (on Carduaceae).

Calea insignis Blake, Quezaltenango, Jan. 31, 1917, *817*.

Calea integrifolia (DC.) Hemsl., Solola, 7000 feet alt., Jan. 28, 1915, *145*; Zunil, Dept. Quezaltenango, Jan. 28, 1917, *790*.

Telia chiefly hypophyllous, crowded and confluent opposite discolored sunken spots 1 1.5 mm. across, early naked, pulvinate, blackish becoming cinereous by germination, ruptured epidermis noticeable; teliospores oblong-cylindric, 16–19 by 45–70 μ, rounded or obtuse above, narrowed below, slightly constricted at septum; wall cinnamon-brown, darker above, 1–2 μ thick, much thicker at apex, 6–12 μ, smooth; pedicel colored like the spore, short.

A short-cycle rust in which the pycnia are probably not formed. It resembles P. *Synedrellae* on the nearly related host *Tridax procumbens*, but with spores half as much larger.

200. PUCCINIA GYMNOLOMIAE Arth. (on Carduaceae).

Gymnolomia microcephala Less., Volcan de Agua, Dept. Sacatépequez, March 4, 1916, II, III, *556*; Mendez, Dept. Guatemala, Feb. 13, 1917, II, III, *861*.

Hymenostephium cordatum (Hook. & Arn.) Blake, San Felipe, Dept. Retalhuleu, Jan. 12, 1917, II, *692*; Colomba, Dept. Quezaltenango, Feb. 3, 1917, II, *828*.

Hymenostephium sp., Antigua, Dept. Sacatépequez, Dec. 28, 1916, II, III, *652*.

A long-cycle rust that probably possesses pycnia and aecia, which, however, have not yet been collected.

201. **Puccinia semota** Jackson & Holway sp. nov. (on Carduaceae).

Gymnolomia subflexuosa Benth., Solola, Jan. 28, 1915, *146*.

Pycnia unseen, probably not formed.

Telia hypophyllous, crowded in small confluent groups 1–2 mm. across, round, 0.3–0.5 mm. in diameter, early naked, pulvinate, dark chestnut-brown, ruptured epidermis inconspicuous, teliospores clavate, 13–18 by 48–58 μ, rounded above, somewhat narrowed below, slightly constricted at septum; wall golden-brown, thin, 1 μ, thickened above, 4–10 μ, smooth; pedicel colorless, short, one third length of spore or less.

A short-cycle rust of the general appearance of *P. Silphii*.

202. PUCCINIA COGNATA Syd. (on Carduaceae).

Verbesina Fraseri Hemsl., Antigua, 5300 feet alt., Dept. Sacatépequez, Jan. 12, 1915, 0, i, ii, III, *73*; Guatemala City, Feb. 8, 1916, II, III, *464*; same, Dec. 20, 1916, II, III, *604*.

Verbesina Holwayi Robinson, Quezaltenango, Jan. 20, 1915, ii, III, *96B*; same, Jan. 17, 1917, III, *738* (with *Coleosporium Verbesinae*).

Verbesina sublobata Benth., San Lucas Toliman, Dept. Solola, Feb. 2, 1915, II, *175A*, *180*.

Verbesina sp., Solola, Jan. 27, 1915, II, *135*; San Lucas Toliman, Dept. Solola, Feb. 2, 1915, II, III, *177*; Mazatenango, Dept. Suchitepequez, Feb. 22, 1916, II, *523*.

A long-cycle species, showing much variability in size and appearance of the teliospores. It was collected by Kellerman on *V. Fraseri*, at Guatemala City, Feb. 1, 1905, ii, III, *4324*, and at Laguna, Lake Amatitlan, January, 1906, ii, III, *5412*, and reported by Kern in Journ. Myc. *l.c.*

203. PUCCINIA AFFINIS Syd. (on Carduaceae).

Verbesina perymenioides Schultz Bip., Guatemala City, Jan. 1, 1915, ii, III, *6*; Laguna, Lake Amatitlan, Feb. 8, 1915, II, *200*.

Neither pycnia nor aecia have yet been seen in connection with this species. It was collected by Kellerman on an undetermined species of Verbesina, appearing very similar to *V. perymenioides*, at Laguna, Lake Amatitlan, Jan. 20, 1906, II, III, *5455*, and reported by Kern in Journ. Myc., under the name of *P. Ximenesiae* Long, a very similar species.

204. PUCCINIA MELAMPODII Diet. & Holw. (on Carduaceae).

Melampodium divaricatum (Rich.) DC., Mazatenango, Dept. Suchitepequez, Feb. 22, 1916, *515*.

A short-cycle leptoform rust, rarely collected. It is known from the type locality in central Mexico, and by a previous collection from Guatemala, seen in the cryptogamic herbarium of the New York Botanical Garden, on the same host, made in Dept. Escuintla, March, 1890, by J. Donnell Smith.

205. PUCCINIA TITHONIAE Diet. & Holw. (on Carduaceae).

Tithonia diversifolia (Hemsl.) A. Gray, San Rafael, Dept. Guatemala, Jan. 10, 1915, II, iii, *65*; same, Jan. 12, 1915, II, *60*; San Felipe, Dept. Retalhuleu, Jan. 12, 1917, II, *701*.

Tithonia rotundifolia (Mill.) Blake (*T. tagetiflora* Desf.), Mazatenango, Feb. 21, 1916, II, III, *514*; San Antonio, Dept. Suchitepequez, Feb. 24, 1916, II, *526*; San Felipe, Dept. Retalhuleu, Jan. 12, 1917, II, *696*; same, Jan. 14, 1917, II, *715*.

Tithonia scaberrima Benth., Quezaltenango, Jan. 16, 1917, II, *729*.

Tithonia tubaeformis Cass., Antigua, Dept. Sacatépequez, Jan. 11, 1915, II, *70*; Guatemala City, Dec. 20, 1916, II, III, *606*.

A long-cycle rust, similar to *P. Helianthi* Schwein, whose first stage is unknown. It was described from Mexico on *T.* "*cubiflora*," an error for *T. tubaeformis*. The first named host has not before been reported. The species was collected by Kellerman on *T. tubaeformis*, at Guatemala City, Feb. 3, 1905, II, III, *4328*, and at Laguna, Lake Amatitlan, Jan. 30, 1906, II, III, *5425*, and reported by Kern in Journ. Myc. *l. c.* No. *5425* was also issued in Kellerm. Fungi Sel. Guat. *18*.

206. PUCCINIA GNAPHALII (Speg.) P. Henn. (on Carduaceae).

Gnaphalium rhodanthum Schultz Bip., Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, *578*.

A long-cycle species, for which the primary stage is not known. Only uredinia have been taken in North America up to the present time.

207. PUCCINIA GNAPHALIATA (Schwein.) Arth. & Bisby (on Carduaceae).

Gnaphalium sp., Guatemala City, Dec. 20, 1916, I, *610*; Antigua, Dept. Sacatépequez, Dec. 28, 1916, I, *655*.

A widespread, long-cycle species, having no uredinia, and not before reported south of Mexico. It is usually listed under the synonymous name *P. investita* Schwein.

208. PUCCINIA MELANTHERAE P. Henn. (on Carduaceae).

Melanthera nivea (L.) Small, Antigua, 5300 feet alt., Dept. Sacatépequez, Jan. 11, 1915, ii, III, *69*.

This long-cycle rust is now first reported from North America. A collection by E. Ule, from Brazil, 1885, is issued in Rab.-Paz. Fung; Europaei *4325*. It probably possesses pycnia and aecia, but they have not yet been seen.

209. **Puccinia cornuta** Jacks. & Holw. sp. nov. (on Carduaceae).

Notoptera brevipes (Robinson) Blake, Guatemala City, Feb. 15, 1916, O, I, III, *493*; same, Feb. 8, 1917, O, I, III, *846* (type).

Pycnia mostly epiphyllous, along the veins on yellowish areas, conspicuous, dark brown, subepidermal, globoid, 75–100 μ in diameter.

Aecia hypophyllous along the veins, scattered on yellowish areas 10–15 mm. across, long cylindric and slightly curved, 0.1 mm. in diameter, 2–3 mm. long, soon breaking up into cylindrical fragments; peridium dirty brown, dehiscent by fragmentation; peridial cells light cinnamon-brown, narrowly rhomboidal, 7–10 by 42–55 μ, somewhat overlapping, the wall 2 μ thick; aeciospores angularly globoid or oblong, 15–26 by 26–40 μ; wall yellowish to pale golden-brown, thin, 1 μ, thicker above up to 7 μ, rather coarsely and closely verrucose above, smooth below.

Telia mostly hypophyllous, arising from the veins and following the aecia on the same discolored areas, giving a dendritic appearance, 0.2–0.5 mm. across, early naked, prominent, chocolate-brown or blackish, ruptured epidermis inconspicuous; teliospores ellipsoid, 23–26 by 32–40 μ, rounded at both ends, slightly or not constricted at septum; wall dark chestnut-brown, 2.5–3 μ thick, closely and prominently verrucose; pedicel colorless, twice to thrice length of spore.

A conspicuous rust of most unusual appearance. The remarkably long, brown aecia look like those of some Gymnosporangium, but show no tendency to slit longitudinally. At first sight they seem like ex-

traneous objects. The dendritic distribution of the blackish, loose telia is also very striking.

210. **Puccinia Trixitis** (Kern & Kellerm.) comb. nov. (on Carduaceae).

Trixis frutescens P. Br., Antigua, 5300 feet alt., Dept. Sacatépequez, Jan. 11, 1915, II, III, *71*; same, March 9, 1916, II, III, *581*; Solola, Jan. 25, 1915, II, iii, *108*; near Santa Maria, Dept. Quezaltenango, Jan. 15, 1917, II, *725*.

This rust was published as *Uredo Trixitis* Kern & Kellerm. founded on a collection made at San Lucas, Dept. Solola, Feb. 15, 1906, Kellerman *5432* (Journ. Mycol. 13: 26. 1907). It was issued as Kellerm. Fungi Sel. Guat. *20*. The beginning stage in the life cycle of the species is yet to be discovered.

211. **Puccinia Schistocarphae** Jacks. & Holw. sp. nov. (on Carduaceae).

Schistocarpha platyphylla Greenm., San Rafael, Dept. Guatemala, Jan. 9, 1915, *42* (type); Volcan de Agua, Dept. Sacatépequez. Jan. 13, 1915, *85*; same, March 7, 1916, *571*.

Schistocarpha sp., Aguas Amargas, Dept. Quezaltenango, Jan. 30, 1917, III, *799*; road between Colomba and Quezaltenango, Feb. 4, 1917, III, *834*.

Telia hypophyllous, crowded over areas 0.5–2 mm. across, early naked, compact, very light yellowish-brown, becoming cinereous by germination, ruptured epidermis inconspicuous; teliospores oblong, 16–22 by 39–55 μ rounded at both ends, or slightly narrowed below, slightly constricted at septum; wall colorless or very light golden-brown, 1–1.5 μ thick, thicker above, 5–9 μ, smooth; pedicel colorless, short.

No pycnia were found with this short-cycle, leptoform rust, and doubtless none are formed.

212. PUCCINIA PROBA Jacks. & Holw. (on Carduaceae).

Zexmenia elegans Schultz Bip., Mulua, between Mazatenango and Retalhuleu, Feb. 26, 1916, O, II, III, *531*; San Felipe, Dept. Retalhuleu, Jan. 12, 1917, II, III, *689*, *698*, *700*; same, Jan. 13, 1917, II, III, *714*.

Zexmenia frutescens (Mill.) Blake, Solola, Jan. 25, 1915, ii, III, *109*; Quirigua, March 22, 1916, O, II_1, II_2, III, *601*

Zexmenia Salvinii Hemsl., Guatemala City, Feb. 8, 1917, II, *847*.

A long-cycle rust, having pycnia, uredinia, and telia. It also occurs in Costa Rica.

In the phanerogamic herbarium at the New York Botanical Garden two additional collections from Guatemala were found, both given as on *Z. costaricensis* (= *Z. frutescens* Blake), one from Cubelquitz, Dept. Alta Vera Paz, Nov. 1900, H. von Türckheim *7746*, and the other from Los Amates, Feb. 6, 1905, C. C. Deam *302*. In the phanerogamic herbarium of the Field Museum sheet no. 194857, bearing *Zexmenia elegans Kellermanii* Greenm., shows this rust, II, III. The collection was made at Los Amates, Jan. 17, 1905, Kellerman *5332*.

213. **Puccinia inaudita** Jacks. & Holw. sp. nov. (on Carduaceae).

Zexmenia leucactis Blake, Escuintla, Feb. 19, 1916, O, I, III, *505*; San Felipe, Dept. Retalhuleu, Jan. 12, 1917, O, I, III, ii, *693* (type); Colomba, Dept. Quezaltenango, Feb. 3, 1917, O, I, ii, iii, *823*.

Zexmenia longipes Benth., Guatemala City, Dec. 23, 1916, O, I, ii, III, *628*.

Pycnia chiefly epiphyllous, usually numerous, on raised spots 0.5–1.5 mm. across, conspicuous, subepidermal, deep-seated, flask-shaped, 125–160 μ broad by 160–190 μ high.

Aecia amphigenous, few in groups opposite or among the pycnia, cylindric, 0.2–0.3 mm. broad by 1–2.5 mm. long; peridium whitish, membranous, becoming deeply lacerate; peridial cells in face view angularly ellipsoid or polyhedral, 20–30 by 45–55 μ, the wall uniformly thin, 1–1.5 μ, very finely and closely verrucose-rugose; aeciospores angularly ellipsoid or globoid, 16–24 by 24–32 μ; wall pale cinnamon-brown, 1.5–2.5 μ thick, coarsely tuberculate with colorless markings giving the appearance of reticulations.

Uredinia hypophyllous, scattered, round or oval, 0.2–0.4 mm. across, early naked, pulverulent, dark cinnamon-brown, ruptured epidermis evident; urediniospores ellipsoid or obovoid, 19–21 by 24–29 μ; wall golden-brown, rather thick, 2 μ, moderately echinulate, the pores 3–4, scattered.

Telia hypophyllous, scattered, round, 0.5–0.8 mm. in diameter, early naked, pulvinate, whitish, ruptured epidermis inconspicuous; teliospores oblong or fusiform-oblong, 16–19 by 42 64 μ, rounded or obtuse above, somewhat narrowed below, constricted at septum; wall colorless, uniformly thin, 1–1.5 μ, the pore of lower cell at septum, smooth; pedicel colorless, fragile, equaling the spore or shorter.

The combination of life cycle and morphological characters in this species makes it especially notable. In gross appearance the very long and delicate aecia together with the small, pale telia easily distinguish it from other forms on Zexmenia and nearly related hosts.

214. PUCCINIA ENCELIAE Diet. & Holw. (on Carduaceae).

Simsia Holwayi Blake, Agua Caliente, Dept. Guatemala, Feb. 10, 1917, II, III, *854*.

Simsia polycephala Benth., Moran, Dept. Amatitlan, Dec. 22, 1916, II, *624*.

Simsia sericea (Hemsl.) Blake (*Encelia sericea* Hemsl.), San Rafael, Dept. Guatemala, Jan. 11, 1915, II, *63*; Volcan de Agua, Dept. Sacatépequez, Jan. 12, 1915, II, *79*; Antigua, Dept. Sacatépequez, March 2, 1916, II, III, *548*.

A long-cycle rust possessing pycnia, uredinia, and telia. It occurs from southern California southward through Mexico and Central America.

215. PUCCINIA DOLORIS Speg. (on Carduaceae).

Erigeron bonariensis leiothecus Blake, San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 8, 1915, *39*.

Erigeron Deamii Robinson, Solola, 7000 feet alt., Jan. 25, 1915, *112*.

Erigeron sp., Guatemala City, Jan. 10, 1917, *686*; Huehuetenango, Jan. 24, 1917, *776*.

A short-cycle species, occurring also in Costa Rica and South America. The teliospores are very small.

216. **Puccinia coreopsidis** Jacks. & Holw. sp. nov. (on Carduaceae).

Coreopsis mexicana (DC.) Hemsl., Guatemala City, Jan. 1, 1915, III, *5*; same, Dec. 21, 1916, ii, III, *613*; San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 9, 1915, ii, III, *52* (type); Solola, Jan. 30, 1915, ii, III, *154*; near Santa Maria, Dept. Quezaltenango, Jan. 15, 1917, ii, III, *725B*.

Uredinia amphigenous, scattered, circular or oval, 0.1–0.3 mm. across, early naked, pulverulent, cinnamon-brown, the ruptured epidermis evident; urediniospores obovoid, 20–24 by 27–32 μ; wall golden, 1–1.5 μ thick, prominently and sparsely echinulate, the pores 2, superequatorial.

Telia amphigenous, scattered, circular or oval, 0.1–0.3 mm. across, early naked, pulverulent, dark chestnut-brown, the ruptured epidermis conspicuous; teliospores ellipsoid or oblong, 23–29 by 35–45 μ, rounded above, rounded or slightly narrowed below, somewhat constricted at septum; wall dark chestnut-brown, 3–4 μ thick, slightly thicker above, 6–7 μ, strongly and sparsely verrucose; pedicel colorless, twice length of spore.

217. PUCCINIA SPEGAZZINII De Toni (on Carduaceae).

Mikania cordifolia (L.f.) Willd. (?), Guatemala City, Feb. 15, 1916, *496* same, Feb. 8, 1917, *843*; Moran, Dept. Amatitlan, Dec. 22, 1916, *622*.

A common short-cycle, leptoform rust of the tropics.

218. PUCCINIA SENECIONICOLA Arth. (on Carduaceae).

Cacalia calotricha Blake, Volcan de Agua, Dept. Sacatépequez, March 7, 1916, II, *570*.

Cacalia sp., Guatemala City, Dec. 23, 1916, I, II, III, *632*; same, Feb. 8, 1917, II, III, *845*; Huehuetenango, Jan. 23, 1917, II, III, *771*; Zunil, Dept. Quezaltenango, Jan. 28, 1917, I, *794*; Colomba, Dept. Quezaltenango, Feb. 3, 1917, I, II, III, *827*; road between Colomba and Quezaltenango, Feb. 4, 1917, II, *835*, *836*.

Senecio sp., Guatemala City, Jan. 5, 1915, II, III, *10*; San Rafael, Dept. Guatemala, Jan. 9, 1915, ii, III, *47*; Quezaltenango, Jan. 20, 1915, II, *93*, *96A*; same, Jan. 16, 1917, II, *728*; same, Jan. 28, 1917, II, *781*; San Felipe, Dept. Retalhuleu, Jan. 13, 1917, II, *702*; Zunil, Dept. Quezaltenango, Jan. 28, 1917, II, *784*.

A species heretofore imperfectly known, and recorded only from Mexico. Three of Professor Holway's collections show aecia, with globoid or broadly ellipsoid spores, 23–30 by 26–35 μ, the wall colorless, 2–3.5 μ thick, coarsely and thickly verrucose. The rust was also collected by Kellerman, on *Senecio petasioides* Greenm., Volcan de Cerro Quemado, Dept. Quezaltenango, Feb. 8, 1906, II, III, *5418*, and at Volcan de Atitlan, Dept. Solola, Feb. 16, 1906, II, III, *5442*, and also on *S. Warszewiczii* A. Br. & Bouché, Volcan de Cerro Quemado, Feb. 8, 1906, II, *5445*, all being reported by Kern in Mycologia *l.c.*

FORM-GENERA

219. UREDO PALLIDA Diet. & Holw. (on Poaceae).

Tripsacum latifolium Hitchc.

This pale, small-spore rust was collected by Kellerman at Agua Caliente, Dept. Guatemala, Jan. 25, 1908, *7802*. It was also found on phanerogamic specimens of the same host from Nicaragua and Salvador, communicated by Mrs. Agnes Chase, from the National Herbarium.

Heretofore the rust has been known only on *T. lanceolatum* Rupr. (erroneously published as *T. dactyloides*) from Mexico, and on *Zea Mays* L. from Porto Rico.

220. **Uredo Triniochloae** Arth. & Holw. sp. nov. (on Poaceae).

Triniochloa stipoides (H.B.K.) Hitchc., San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 10, 1915, *59*.

Uredinia chiefly epiphyllous, numerous, small, elliptic, 0.2–0.5 mm. long, soon naked, yellowish, pulverulent, ruptured epidermis inconspicuous; paraphyses numerous, erect, clavate or capitate, unusually large, 10–29 by 58–98 μ, the wall yellowish, uniformly thin, 1–2 μ, sometimes slightly thicker above; urediniospores ellipsoid or obovoid, 16–19 by 19–26 μ; wall yellowish to pale cinnamon-brown, thin, about 1 μ, finely and closely echinulate, the pores obscure.

The species is remarkable for its large paraphyses.

221. **Uredo Zeugitis** Arth. & Holw. sp. nov. (on Poaceae).

Zeugites Hartwegi Fourn., San Rafael, 7000 feet alt., Dept. Guatemala, Jan. 9, 1915, *49*.

Uredinia chiefly hypophyllous, scattered, elliptic, small, 0.3–0.5 mm. long, rather tardily naked, cinnamon-brown, ruptured epidermis evident; urediniospores broadly ellipsoid, 19–21 by 23–26 μ; wall cinnamon-brown, moderately thick, 1.5–2.5 μ, finely and closely echinulate, the pores 3, sometimes 4, equatorial.

The host belongs to the tribe Festucaceae, in which no rust identical with this one has been seen.

222. UREDO RUBESCENS Arth. (on Artocarpaceae).

Dorstenia Contrajerva L., Palin, Dept. Amatitlan, Dec. 24, 1916, *634*.

Dorstenia Houstoni L., Mazatenango, Feb. 22, 1916, *520*; San Felipe, Dept. Retalhuleu, Jan. 13, 1917, *708*.

The first record for this rust outside of Porto Rico. No telia have yet been discovered.

223. **Uredo Fuchsiae** Arth. & Holw. sp. nov. (on Onagraceae).

Fuchsia splendens Zucc. (?), Volcan de Agua, Dept. Sacatépequez, March 7, 1916, *563* (type).

Lopezia hirsuta Jacq., Antigua, Dept. Sacatépequez, Dec. 28, 1916, *649* (with some *Puccinia Fuchsiae*).

Uredinia hypophyllous, in small irregular groups 0.5–3 mm. across, round, 0.1–0.2 mm. in diameter, long covered by the epidermis, pulverulent, pale yellow, ruptured epidermis evident; peridium hemispheric, delicate, opening at first by a small pore, later breaking away and exposing the spores, the peridial cells rectangular or rhombic, abutted, the walls colorless, thin, 1 μ, not thickened or sculptured at

the ostiole; urediniospores ellipsoid, 13–16 by 18–24 μ; wall colorless, moderately thick, 1–2 μ, rather inconspicuously echinulate, the pores obscure.

The form of the sorus in this species indicates that the rust may belong under the genus Pucciniastrum. The flat hymenium, the structure of the peridium and its behavior in dehiscence, the pale spores with thin wall and obscure pores, are all features strongly suggesting Pucciniastrum. The spores, as in species of that genus, appear sessile, but fall away as others do that have been found to be primitively catenulate. It is probably a species closely related to *Pucciniastrum pustulatum* (Pers.) Diet., and *P. Circaeae* (Thüm). Speg.; both of which are on Onagraceous hosts.

224. UREDO PERIBUYENSIS Speg. (on Polygalaceae).

Polygala americana Mill., Guatemala City, Jan. 8, 1917, *682*.

This unconnected uredinial form has an applanate sorus, without paraphyses, and agrees well with the original South American collection. The type is published as on Monninia sp., but a collection, labeled otherwise as published for the type, is given as on Polygala. A third collection, made by C. G. Pringle and communicated by W. G. Farlow, on *P. acicularis*, Santa Eulalia Mts., Chihuahua, Mexico, Nov. 15, 1886, can also be placed under this name, although the spores are more variable in size than either of the other two collections, and have slightly thicker walls.

225. **Uredo Rondeletiae** Arth. & Holw. sp. nov. (on Rubiaceae).

Rondeletia cordata Benth., Guatemala City, Feb. 8, 1917, *842*.

Uredinia hypophyllous, scattered, round, 0.1–0.4 mm. across, early naked, pulverulent, cinnamon-brown, ruptured epidermis evident; peridium and paraphyses none; urediniospores obovoid-reniform, 13–21 by 23–29 μ; wall cinnamon-brown, thin, 1 μ, closely echinulate, the pores obscure.

226. UREDO PLUCHEAE Syd. (on Carduaceae).

Pluchea odorata Cass.

A collection of this rust was made by Kellerman, at Amatitlan, Jan. 25, 1906, *5388*, and reported under the synonymous name of *U. biocellata* Arth. in Journ. Myc. *l.c.*, and thus issued in Kellerm. Fungi Sel. Guat. *19*. The species is also known from southern Florida and from the West Indies.

227. PERIDERMIUM GUATEMALENSE Arth. & Kern (on Pinaceae).

Pinus filifolia Lindl.

Collections were made by Kellerman, at Antigua, Dept. Sacatépequez, Feb. 13, 1905, *4624*, *5324*, *5355*, and reported by Kern in Journ. Myc. *l.c.*, under the name *P. gracile*. No collection of the species other than these is known.

228. AECIDIUM LORANTHI Thüm. (on Loranthaceae).

Psittacanthus calyculatus (DC.) G. Don.

A specimen was taken, Feb. 27, 1902, by William Trelease in Guatemala, no locality given, and reported by Kern in Mycologia, *l.c.* The specimen was seen in the herbarium of the Missouri Botanical Garden, and was labeled "*Aecidium Cookeanum*? on Loranthus." Dr. Trelease was consulted regarding the host, and under date of January 24, 1916, replied: "My impression is that I got specimens of the orange-flowered mistletoe. . . . Your Guatemalan rust is pretty clearly on a Psittacanthus, and very likely on *P. calyculatus*." Type collections of mistletoe rusts have not been available for comparison, but as near as can be told by the meager description this collection should be referred to *A. Loranthi* Thüm. The species has much larger aeciospores than in *Uromyces ornatipes* Arth. It may belong to one of the species published for South America, but no suitable material for comparison is at hand.

229. **Aecidium singulare** (Diet. & Holw.) comb. nov. (on Malpighiaceae).

Byrsonima crassifolia (L.) H.B.K.

The rust was collected by Kellerman at Sierra de las Minas, 2000 feet alt., opposite El Rancho, Dept. Baja Vera Paz, March 10, 1905, *4325*, and reported by Kern in Journ. Myc. *l.c.*, as *A. Byrsonimae* K. & K., and issued in Kellerm. Fungi Sel. Guat. *11*. It was earlier published from Mexico as *Endophyllum singulare* Diet. & Holw. It is very similar to *A. Brysonimatis* P. Henn. from Brazil, and may be identical with it. The species is also known from Nicaragua. The morphological appearance suggests a possible connection with a Cronartium, as its aecial stage.

230. AECIDIUM ALBICANS Arth. & Holw. (on Euphorbiaceae).

Phyllanthus acuminatus Vahl, Escuintla, Feb. 19, 1916, I, *504*; San Felipe, Dept. Retalhuleu, Jan. 13, 1917, O, I, *709*.

The same rust occurs in Costa Rica and on the same host.

231. AECIDIUM GUATEMALENSIS Kern & Kellerm. (on Heliotropaceae).
Heliotropium indicum L.

The type collection was made by Kellerman, at Gualan, 400 feet alt., Dept. Zacapa, March 12, 1905, *4326*, and was described by Kern in Journ. Myc. *l.c.* No additional information has come to hand since the original collection was made.

232. **Aecidium seriatum** sp. nov. (on Euphorbiaceae).
Eumecanthus lancifolius (Schlecht.) Millsp. (*Euphorbia lancifolia* Schlecht.).

Pycnia chiefly hypophyllous, numerous, in groups 1–3 mm. across, punctiform, noticeable, subcuticular, hemispherical, 80–115 μ in diameter by 40–75 μ high.

Aecia hypophyllous, numerous, in more or less evident concentric circles surrounding the central group of pycnia, on spots 1.5–2 cm. across, yellowish below, reddish above, cupulate, 0.3–0.5 mm. in diameter, low, erect; peridium white, the margin irregularly torn; peridial cells nearly rectangular in radial longitudinal section, 15–22 by 18–26 μ, slightly overlapping, the outer wall smooth, transversely striate, 6–8 μ thick, the inner wall closely and prominently verrucose, 3–4 μ thick; aeciospores irregularly globoid, 12–18 μ in diameter; wall colorless, about 1 μ thick, very finely and inconspicuously verrucose, often appearing smooth.

The rust has the appearance of a heteroecious form, although the subcuticular pycnia indicate that it may be an autoecious form. The name is founded on a collection sent from the herbarium of the National Museum, made by H. Pittier, on *Eumecanthus lancifolius* (Schlecht.) Millsp. (*Euphorbia lancifolia* Schlecht.), between Cajval and Chimente, along Cahabor Rio, alt. 200 meters, Dept. Alta Vera Paz, March 4, 1905, *237*.

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